DESCRIPTION

ENZYMATIC NUCLEIC ACID TREATMENT OF DISEASES OR CONDITIONS RELATED TO HEPATITIS C VIRUS INFECTION

This patent application is a continuation-in-part of Blatt et al., USSN 09/504,231, filed February 15, 2000, which is a continuation-in-part of Blatt et al., USSN 09/274,553, filed March 22, 1999 and Blatt et al., USSN 09/257,608, filed February 24, 1999, which both claim the benefit of Blatt et al., USSN 60/100,842, filed September 18, 1998, and McSwiggen et al., USSN 60/083,217 filed April 27, 1998, all of these earlier applications are entitled "ENZYMATIC NUCLEIC ACID TREATMENT OF DISEASES OR CONDITIONS RELATED TO HEPATITIS C VIRUS INFECTION". Each of these applications are hereby incorporated by reference herein in their entirety including the drawings.

Background Of The Invention

This invention relates to methods and reagents for the treatment of diseases or conditions relating to the hepatitis C virus (HCV) infection.

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

In 1989, the HCV was determined to be an RNA virus and was identified as the causative agent of most non-A non-B viral Hepatitis (Choo *et al., Science.* 1989; 244:359-362). Unlike retroviruses such as HIV, HCV does not go though a DNA replication phase and no integrated forms of the viral genome into the host chromosome have been detected (Houghton *et al., Hepatology* 1991;14:381-388). Rather, replication of the coding (plus) strand is mediated by the production of a replicative (minus) strand leading to the generation of several copies of plus strand HCV RNA. The genome consists of a single, large, open-reading frame that is translated into a polyprotein (Kato *et al., FEBS Letters.* 1991; 280: 325-328). This polyprotein subsequently undergoes post-translational cleavage, producing several viral proteins (Leinbach *et al., Virology.* 1994: 204:163-169).

Examination of the 9.5-kilobase genome of HCV has demonstrated that the viral nucleic acid can mutate at a high rate (Smith *et al., Mol. Evol.* 1997 45:238-246). This rate of

mutation has led to the evolution of several distinct genotypes of HCV that share approximately 70% sequence identity (Simmonds *et al., J. Gen. Virol.* 1994; 75 :1053-1061). It is important to note that these sequences are evolutionarily quite distant. For example, the genetic identity between humans and primates such as the chimpanzee is approximately 98%. In addition, it has been demonstrated that an HCV infection in an individual patient is composed of several distinct and evolving quasi-species that have 98% identity at the RNA level. Thus, the HCV genome is hypervariable and continuously changing. Although the HCV genome is hypervariable, there are 3 regions of the genome that are highly conserved. These conserved sequences occur in the 5' and 3' non-coding regions as well as the 5'-end of the core protein coding region and are thought to be vital for HCV RNA replication as well as translation of the HCV polyprotein. Thus, therapeutic agents that target these conserved HCV genomic regions may have a significant impact over a wide range of HCV genotypes. Moreover, it is unlikely that drug resistance will occur with ribozymes specific to conserved regions of the HCV genome. In contrast, therapeutic modalities that target inhibition of enzymes such as the viral proteases or helicase are likely to result in the selection for drug resistant strains since the RNA for these viral encoded enzymes is located in the hypervariable portion of the HCV genome.

After initial exposure to HCV, the patient will experience a transient rise in liver enzymes, which indicates that inflammatory processes are occurring (Alter *et al.*, IN: Seeff LB, Lewis JH, eds. *Current Perspectives in Hepatology*. New York: Plenum Medical Book Co; 1989:83-89). This elevation in liver enzymes will occur at least 4 weeks after the initial exposure and may last for up to two months (Farci *et al., New England Journal of Medicine.* 1991:325:98-104). Prior to the rise in liver enzymes, it is possible to detect HCV RNA in the patient's serum using RT-PCR analysis (Takahashi *et al., American Journal of Gastroenterology.* 1993:88:2:240-243). This stage of the disease is called the acute stage and usually goes undetected since 75% of patients with acute viral hepatitis from HCV infection are asymptomatic. The remaining 25% of these patients develop jaundice or other symptoms of hepatitis.

Acute HCV infection is a benign disease, however, and as many as 80% of acute HCV patients progress to chronic liver disease as evidenced by persistent elevation of serum alanine aminotransferase (ALT) levels and by continual presence of circulating HCV RNA (Sherlock, *Lancet* 1992; 339:802). The natural progression of chronic HCV infection over a 10 to 20 year period leads to cirrhosis in 20 to 50% of patients (Davis *et al., Infectious Agents and Disease* 1993;2:150:154) and progression of HCV infection to hepatocellular carcinoma has been well

documented (Liang *et al., Hepatology.* 1993; 18:1326-1333; Tong *et al., Western Journal of Medicine*, 1994; Vol. 160, No. 2: 133-138). There have been no studies that have determined sub-populations that are most likely to progress to cirrhosis and/or hepatocellular carcinoma, thus all patients have an equal risk of progression.

It is important to note that the survival for patients diagnosed with hepatocellular carcinoma is only 0.9 to 12.8 months from initial diagnosis (Takahashi *et al., American Journal of Gastroenterology.* 1993:88:2:240-243). Treatment of hepatocellular carcinoma with chemotherapeutic agents has not proven effective and only 10% of patients will benefit from surgery due to extensive tumor invasion of the liver (Trinchet *et al., Presse Medicine.* 1994:23:831-833). Given the aggressive nature of primary hepatocellular carcinoma, the only viable treatment alternative to surgery is liver transplantation (Pichlmayr *et al., Hepatology.* 1994:20:33S-40S).

Upon progression to cirrhosis, patients with chronic HCV infection present with clinical features, which are common to clinical cirrhosis regardless of the initial cause (D'Amico *et al., Digestive Diseases and Sciences.* 1986;31:5: 468-475). These clinical features may include: bleeding esophageal varices, ascites, jaundice, and encephalopathy (Zakim D, Boyer TD. Hepatology a textbook of liver disease. Second Edition Volume 1. 1990 W.B. Saunders Company. Philadelphia). In the early stages of cirrhosis, patients are classified as compensated meaning that although liver tissue damage has occurred, the patient's liver is still able to detoxify metabolites in the blood-stream. In addition, most patients with compensated liver disease are asymptomatic and the minority with symptoms report only minor symptoms such as dyspepsia and weakness. In the later stages of cirrhosis, patients are classified as decompensated meaning that their ability to detoxify metabolites in the bloodstream is diminished and it is at this stage that the clinical features described above will present.

In 1986, D'Amico *et al.* described the clinical manifestations and survival rates in 1155 patients with both alcoholic and viral associated cirrhosis (D'Amico *supra*). Of the 1155 patients, 435 (37%) had compensated disease although 70% were asymptomatic at the beginning of the study. The remaining 720 patients (63%) had decompensated liver disease with 78% presenting with a history of ascites, 31% with jaundice, 17% had bleeding and 16% had encephalopathy. Hepatocellular carcinoma was observed in six (.5%) patients with compensated disease and in 30 (2.6%) patients with decompensated disease.

Over the course of six years, the patients with compensated cirrhosis developed clinical features of decompensated disease at a rate of 10% per year. In most cases, ascites was the first presentation of decompensation. In addition, hepatocellular carcinoma developed in 59 patients who initially presented with compensated disease by the end of the six-year study.

With respect to survival, the D'Amico study indicated that the five-year survival rate for all patients on the study was only 40%. The six-year survival rate for the patients who initially had compensated cirrhosis was 54% while the six-year survival rate for patients who initially presented with decompensated disease was only 21%. There were no significant differences in the survival rates between the patients who had alcoholic cirrhosis and the patients with viral related cirrhosis. The major causes of death for the patients in the D'Amico study were liver failure in 49%; hepatocellular carcinoma in 22%; and, bleeding in 13% (D'Amico *supra*).

Chronic Hepatitis C is a slowly progressing inflammatory disease of the liver, mediated by a virus (HCV) that can lead to cirrhosis, liver failure and/or hepatocellular carcinoma over a period of 10 to 20 years. In the US, it is estimated that infection with HCV accounts for 50,000 new cases of acute hepatitis in the United States each year (NIH Consensus Development Conference Statement on Management of Hepatitis C March 1997). The prevalence of HCV in the United States is estimated at 1.8% and the CDC places the number of chronically infected Americans at approximately 4.5 million people. The CDC also estimates that up to 10,000 deaths per year are caused by chronic HCV infection. The prevalence of HCV in the United States is estimated at 1.8% and the CDC places the number of chronically infected Americans at approximately 4.5 million people. The CDC also estimates that up to 10,000 deaths per year are caused by chronic HCV infection.

Numerous well controlled clinical trials using interferon (IFN-alpha) in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (range 40% to 70%) of patients by the end of 6 months of therapy (Davis *et al., New England Journal of Medicine* 1989; 321:1501-1506; Marcellin *et al., Hepatology*. 1991; 13:393-397; Tong *et al., Hepatology* 1997:26:747-754; Tong *et al., Hepatology* 1997 26(6): 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a "durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 to 25%.

In recent years, direct measurement of the HCV RNA has become possible through use of either the branched-DNA or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis. In general, the RT-PCR methodology is more sensitive and leads to more accurate assessment of the clinical course (Tong *et al., supra*). Studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients will have a loss of HCV RNA by the end of therapy (Marcellin *et al., supra*). However, as with the ALT endpoint, about 50% of the patients relapse six months following cessation of therapy resulting in a durable virologic response of only 12% (Marcellin *et al., supra*). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25% (NIH consensus statement: 1997). Thus, standard of care for treatment of chronic HCV infection with type 1 interferon is now 48 weeks of therapy using changes in HCV RNA concentrations as the primary assessment of efficacy (Hoofnagle *et al., New England Journal of Medicine* 1997; 336(5) 347-356).

Side effects resulting from treatment with type 1 interferons can be divided into four general categories, which include 1. Influenza-like symptoms; 2. Neuropsychiatric; 3. Laboratory abnormalities; and, 4. Miscellaneous (Dusheiko *et al., Journal of Viral Hepatitis*, 1994:1:3-5). Examples of influenza-like symptoms include: fatigue, fever; myalgia; malaise; appetite loss; tachycardia; rigors; headache and arthralgias. The influenza-like symptoms are usually short-lived and tend to abate after the first four weeks of dosing (Dushieko *et al., supra*). Neuropsychiatric side effects include: irritability, apathy; mood changes; insomnia; cognitive changes and depression. The most important of these neuropsychiatric side effects is depression and patients who have a history of depression should not be given type 1 interferon. Laboratory abnormalities include; reduction in myeloid cells including granulocytes, platelets and to a lesser extent red blood cells. These changes in blood cell counts rarely lead to any significant clinical sequellae (Dushieko *et al., supra*). In addition, increases in triglyceride concentrations and elevations in serum alanine and aspartate aminotransferase concentration have been observed. Finally, thyroid abnormalities have been reported. These thyroid abnormalities are usually reversible after cessation of interferon therapy and can be controlled with appropriate medication while on therapy. Miscellaneous side effects include nausea; diarrhea; abdominal and back pain; pruritus; alopecia; and rhinorrhea. In general, most side effects will abate after 4 to 8 weeks of therapy (Dushieko *et al., supra*).

Welch *et al., Gene Therapy* 1996 3(11): 994-1001 describe *in vitro* an *in vivo* studies with two vector expressed hairpin ribozymes targeted against hepatitis C virus.

Sakamoto *et al., J. Clinical Investigation* 1996 98(12): 2720-2728 describe intracellular cleavage of hepatitis C virus RNA and inhibition of viral protein translation by certain vector expressed hammerhead ribozymes.

Lieber *et al., J. Virology* 1996 70(12): 8782-8791 describe elimination of hepatitis C virus RNA in infected human hepatocytes by adenovirus-mediated expression of certain hammerhead ribozymes.

Ohkawa *et al.,* 1997, *J. Hepatology*, 27; 78-84, describe *in vitro* cleavage of HCV RNA and inhibition of viral protein translation using certain *in vitro* transcribed hammerhead ribozymes.

Barber *et al.,* International PCT Publication No. *WO 97/32018*, describe the use of an adenovirus vector to express certain anti-hepatitis C virus hairpin ribozymes.

Kay *et al.,* International PCT Publication No. *WO 96/18419*, describe certain recombinant adenovirus vectors to express anti-HCV hammerhead ribozyme.

Yamada *et al.,* Japanese Patent Application No. *JP 07231784* describe a specific poly-(L)-lysine conjugated hammerhead ribozyme targeted against HCV.

Draper, U.S. Patent Nos. 5,610,054 and 5,869,253, describe enzymatic nucleic acid molecules capable of inhibiting replication of HCV.

SUMMARY OF THE INVENTION

This invention relates to ribozymes, or enzymatic nucleic acid molecules, directed to cleave RNA species of hepatitis C virus (HCV) and/or encoded by the HCV. In particular, applicant describes the selection and function of ribozymes capable of specifically cleaving HCV RNA. Such ribozymes may be used to treat diseases associated with HCV infection.

Due to the high sequence variability of the HCV genome, selection of ribozymes for broad therapeutic applications would likely involve the conserved regions of the HCV genome. Specifically, the present invention describes hammerhead ribozymes that would cleave in the conserved regions of the HCV genome. A list of the thirty hammerhead ribozymes derived from the conserved regions (5'- Non Coding Region (NCR), 5'- end of core protein coding region, and 3'- NCR) of the HCV genome is shown in **Table IV**. In general, Applicant has found that enzymatic nucleic acid molecules that cleave sites located in the 5' end of the HCV genome would block translation while ribozymes that cleave sites located in the 3' end of the genome would block RNA replication. Approximately 50 HCV isolates have been identified and a sequence alignment of these isolates from genotypes 1a, 1b, , 2a, 2b, 2c, 3a, 3b, 4a, 5a, and 6 was performed. These alignments were used by the Applicant to identify 30 hammerhead ribozymes sites within regions highly conserved between genotypes. Twenty-three ribozyme sites were identified in regions of greatest homology within the conserved region. Therefore, one ribozyme can be designed to cleave all the different isolates of HCV. According to the Applicant, ribozymes designed against conserved regions of various HCV isolates will enable efficient inhibition of HCV replication in diverse patient populations and may ensure the effectiveness of the ribozymes against HCV quasi species which evolve due to mutations in the non-conserved regions of the HCV genome.

In another preferred embodiment, the invention features the use of an enzymatic nucleic acid molecule, preferably in the hammerhead, NCH motif (Inozyme), G-cleaver, amberzyme, zinzyme and/or DNAzyme motif, to inhibit the expression of HCV RNA.

In yet another preferred embodiment, the invention features the use of an enzymatic nucleic acid molecule, preferably in the hammerhead, Inozyme, G-cleaver, amberzyme, zinzyme and/or DNAzyme motif, to inhibit the expression of HCV minus strand RNA.

By "inhibit" it is meant that the activity of HCV or level of RNAs or equivalent RNAs encoding one or more protein subunits of HCV is reduced below that observed in the absence of the nucleic acid molecules of the invention. In one embodiment, inhibition with enzymatic nucleic acid molecule preferably is below that level observed in the presence of an enzymatically inactive or attenuated molecule that is able to bind to the same site on the target RNA, but is unable to cleave that RNA. In another embodiment, inhibition of HCV genes with the nucleic acid molecule of the instant invention is greater than in the presence of the nucleic acid molecule than in its absence.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave target RNA. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. The nucleic acids may be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not meant to be limiting and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it have a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving activity to the molecule (Cech *et al.*, U.S. Patent No. 4,987,071; Cech *et al.*, 1988, *JAMA*).

By "nucleic acid molecule" as used herein is meant a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and may comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

By "enzymatic portion" or "catalytic domain" is meant that portion/region of the ribozyme essential for cleavage of a nucleic acid substrate (for example, see Figure 1).

By "substrate binding arm" or "substrate binding domain" is meant that portion/region of a ribozyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figure 1 and 3. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme and target RNA together through complementary base-pairing interactions. The ribozyme of the invention may have binding arms that are contiguous or non-contiguous and may be of varying lengths. The length of the binding arm(s) are preferably greater than or equal to four nucleotides; specifically 12-100 nucleotides; more specifically 14-24 nucleotides long. If two binding arms are chosen, the design is such that the length of the binding arms are symmetrical (*i.e.*, each of the binding arms is of the same length; *e.g.*, five and five nucleotides, six and six nucleotides or seven and seven nucleotides long) or asymmetrical (*i.e.*, the binding arms are of different length; *e.g.*, six and three nucleotides; three and six nucleotides long; four and five nucleotides long; four and six nucleotides long; four and seven nucleotides long; and the like).

By "Inozyme" motif is meant, an enzymatic nucleic acid molecule comprising a motif as described in Ludwig *et al.*, USSN No. 09/406,643, filed September 27, 1999, entitled "COMPOSITIONS HAVING RNA CLEAVING ACTIVITY", and International PCT publication Nos. WO 98/58058 and WO 98/58057, all incorporated by reference herein in their entirety including the drawings.

By "G-cleaver" motif is meant, an enzymatic nucleic acid molecule comprising a motif as described in Eckstein *et al.*, International PCT publication No. WO 99/16871, incorporated by reference herein in its entirety including the drawings.

By "zinzyme" motif is meant, a class II enzymatic nucleic acid molecule comprising a motif as described in Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein in its entirety including the drawings.

By "amberzyme" motif is meant, a class I enzymatic nucleic acid molecule comprising a motif as described in Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein in its entirety including the drawings.

By 'DNAzyme' is meant, an enzymatic nucleic acid molecule that does not require the presence of a 2'-OH group for its activity. In particular embodiments, the enzymatic nucleic

acid molecule may have an attached linker(s) or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups.

By "sufficient length" is meant an oligonucleotide of greater than or equal to 3 nucleotides that is of a length great enough to provide the intended function under the expected condition. For example, for binding arms of enzymatic nucleic acid "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected binding conditions. Preferably, the binding arms are not so long as to prevent useful turnover.

By "stably interact" is meant, interaction of the oligonucleotides with target nucleic acid (*e.g.*, by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions).

By "equivalent" RNA to HCV is meant to include those naturally occurring RNA molecules associated with HCV infection in various animals, including human, rodent, primate, rabbit and pig. The equivalent RNA sequence also includes in addition to the coding region, regions such as 5'-untranslated region, 3'-untranslated region, introns, intron-exon junction and the like.

By "homology" is meant the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

In one of the preferred embodiments of the inventions herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis d virus, group I intron, group II intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Dreyfus, *supra,* Rossi *et al.*, 1992, *AIDS Research and Human Retroviruses* 8, 183; Hairpin motifs are described by Hampel *et al.*, EP0360257, Hampel and Tritz, 1989 *Biochemistry* 28, 4929, Feldstein *et al.*, 1989, *Gene* 82, 53, Haseloff and Gerlach, 1989, *Gene*, 82, 43, and Hampel *et al.*, 1990 *Nucleic Acids Res.* 18, 299; The hepatitis d virus motif is described by Perrotta and Been, 1992 *Biochemistry* 31, 16; The RNaseP motif is described by Guerrier-Takada *et al.*, 1983 *Cell* 35, 849; Forster and Altman, 1990, *Science* 249, 783; Li and Altman, 1996, *Nucleic Acids Res.* 24, 835; *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 *Proc. Natl. Acad. Sci.*

USA 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799; Guo and Collins, 1995, *EMBO. J.* 14, 363); Group II introns are described by Griffin *et al.*, 1995, *Chem. Biol.* 2, 761; Michels and Pyle, 1995, *Biochemistry* 34, 2965; Pyle *et al.*, International PCT Publication No. WO 96/22689; The Group I intron is described by Cech *et al.*, U.S. Patent 4,987,071; and the DNAzyme motif is described by Chartrand *et al.*, 1995, *Nucleic Acids Research* 23, 4092; Santoro *et al.*, 1997, *PNAS* 94, 4262. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., ribozyme cleavage, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, *CSH Symp. Quant. Biol. LII* pp.123-133; Frier et al., 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner et al., 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

In a preferred embodiment, the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding HCV proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of HCV activity in a cell or tissue.

By "related" is meant that the inhibition of HCV RNAs and thus reduction in the level respective viral activity will relieve to some extent the symptoms of the disease or condition.

In preferred embodiments, the ribozymes have binding arms which are complementary to the target sequences in **Tables IV-VIII and X.** Examples of such ribozymes are also shown in **Tables IV-X.** Examples of such ribozymes consist essentially of sequences defined in these tables. Other sequences may be present which do not interfere with such cleavage.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage. Thus, a core region may, for example, include one or more loop or stem-loop structures, which do not prevent enzymatic activity. "X" in the sequences in **Tables V-VIII** can be such a loop. A core sequence for a hammerhead ribozyme can be CUGAUGAG X CGAA where X=GCCGUUAGGC or other stem II region known in the art.

Thus, in a first aspect, the invention features ribozymes that inhibit gene expression and/or viral replication. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, HCV gene expression and/or replication is inhibited.

In a preferred embodiment, ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation in biopolymers.

In another preferred embodiment, the ribozyme is administered to the site of HCV activity (e.g., hepatocytes) in an appropriate liposomal vehicle.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit HCV activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review see Couture and Stinchcomb, 1996, *TIG.*, 12, 510). In another aspect of the invention, ribozymes that cleave target molecules and inhibit viral replication are expressed from transcription units inserted into DNA, RNA, or viral vectors. Preferably, the recombinant vectors capable of expressing the ribozymes are locally delivered as described above, and transiently persist in smooth muscle cells. However, other mammalian cell vectors that direct the expression of RNA may be used for this purpose.

By "patient" is meant an organism which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which enzymatic nucleic acid molecules can be administered. Preferably, a patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell may be present in an organism which may be a human but is preferably a non-human multicellular organism, e.g., birds, plants and mammals such as cows, sheep, apes, monkeys, swine, dogs, and cats. The cell may be prokaryotic (*e.g.* bacterial cell) or eukaryotic (*e.g.*, mammalian or plant cell).

By RNA is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position (eg; 2'-OH) of a β-D-ribo-furanose moiety.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

These ribozymes, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with HCV levels, the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art.

In a further embodiment, the described molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat liver failure, hepatocellular carcinoma, cirrhosis, and/or other disease states associated with HCV infection. Additional known therapeutic agents are those comprising antivirals, interferon, and/or antisense compounds.

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 shows the secondary structure model for seven different classes of enzymatic nucleic acid molecules. Arrow indicates the site of cleavage. -------- indicate the target sequence. Lines interspersed with dots are meant to indicate tertiary interactions. - is meant to indicate base-paired interaction. **Group I Intron**: P1-P9.0 represent various stem-loop structures (Cech *et al.*, 1994, *Nature Struc. Bio.*, 1, 273). **RNase P (M1RNA)**: EGS represents external guide sequence (Forster *et al.*, 1990, *Science*, 249, 783; Pace *et al.*, 1990, *J. Biol. Chem.*, 265, 3587). **Group II Intron:** 5'SS means 5' splice site; 3'SS means 3'-splice site; IBS means intron binding site; EBS means exon binding site (Pyle *et al.*, 1994, *Biochemistry*, 33, 2716). **VS RNA:** I-VI are meant to indicate six stem-loop structures; shaded regions are meant to indicate tertiary interaction (Collins, International PCT Publication No. WO 96/19577). **HDV Ribozyme: :** I-IV are meant to indicate four stem-loop structures (Been *et al.*, US Patent No. 5,625,047). **Hammerhead Ribozyme: :** I-III are meant to indicate three stem-loop structures; stems I-III can be of any length and may be symmetrical or asymmetrical (Usman *et al.*, 1996, *Curr. Op. Struct. Bio.*, 1, 527). **Hairpin Ribozyme:** Helix 1, 4 and 5 can be of any length; Helix 2 is between 3 and 8 base-pairs long; Y is a pyrimidine; Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, *i.e.*, m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, r is 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate.

"q" is ≥2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond. (Burke *et al.*, 1996, *Nucleic Acids & Mol. Biol.*, 10, 129; Chowrira *et al.*, US Patent No. 5,631,359).

Figure 2 is a graph displaying the ability of ribozymes targeting various sites within the conserved 5' HCV UTR region to cleave the transcripts made from several genotypes.

Figure 3 is a schematic representation of the Dual Reporter System utilized to demonstrate ribozyme-mediated reduction of luciferase activity in cell culture.

Figure 4 is a graph demonstrating the ability of ribozymes to reduce luciferase activity in OST-7 cells.

Figure 5 is a graph demonstrating the ability of ribozymes targeting sites HCV.5-313 and HCV.5-318, to reduce luciferase activity in OST-7 cells compared to their inactive controls.

Figure 6A is a bar graph demonstrating the effect of ribozyme treatment on HCV-Polio virus (PV) replication. HeLa cells in 96-well plates were infected with HCV-PV at a multiplicity of infection (MOI) of 0.1. Virus inoculum was then replaced with media containing 5% serum and ribozyme or control (200nM), as indicated, complexed to a cationic lipid. After 24 hours, cells were lysed 3 times by freeze/thaw and virus was quantified by plaque assay. Scrambled control (SAC), binding control (BAC), 3 P=S ribozymes, and 4 P=S ribozymes are indicated. Plaque forming units (pfu)/ml are shown as the mean of triplicate samples + standard deviation (S.D.).

Figure 6B is a bar graph demonstrating the effect of ribozyme treatment on wild type PV replication. HeLa cells in 96-well plates were infected with *wild type* PV at an MOI = 0.05 for 30 minutes. All ribozymes contained 4P=S in (**B**). Plaque forming units (pfu)/ml are shown as the mean of triplicate samples + standard deviation (S.D.).

Figure 7 is a schematic representation of various hammerhead ribozyme constructs targeted against HCV RNA.

Figure 8 is a graph demonstrating the effect of site 183 ribozyme treatment on a single round of HCV-PV infection. HeLa cells were infected with HCV-PV at an MOI = 5 for 30 minutes prior to treatment with ribozymes or control. Cells were lysed after 6, 7, or 8 hours and virus was quantified by plaque assay. Ribozyme binding arm/stem II formats (7/4, 7/3, 6/4, 6/3) and scrambled control (SAC, 7/4 format) are indicated. All contained 4P=S stabilization. Results in pfu/ml are shown as the median of duplicate samples ± range.

Figure 9 shows the secondary structure models of three ribozyme motifs described in this application.

Figure 10 shows the activity of anti-HCV ribozymes in combination with Interferon. Results in pfu/ml are shown as the median of duplicate samples ± range. BAC, binding attenuated control molecule; IF, interferon; Rz, hammerhead ribozyme targeted to HCV site 183; pfu, plaque forming unit.

Figure 11 is a bar graph demonstrating the effect of ribozyme treatment on HCV-Polio virus (PV) replication using anti-HCV ribozymes directed against sites in the HCV minus strand. Both RPI motif I (Hammerhead) and motif II (Inozyme) ribozymes are represented. HeLa cells in 96-well plates were infected with HCV-PV at a multiplicity of infection (MOI) of 0.1. Virus inoculum was then replaced with media containing 5% serum and ribozyme or control (200nM), as indicated, complexed to a cationic lipid. After 24 hours, cells were lysed 3 times by freeze/thaw and virus was quantified by plaque assay. Scrambled control (SAC) and ribozymes targeting different sites are indicated. Plaque forming units (pfu)/ml are shown as the mean of triplicate samples + standard deviation (S.D.). Ribozymes used in this study are shown in **Table X**.

Figure 12 is a bar graph demonstrating the effect of ribozyme treatment on HCV-Polio virus (PV) replication using anti-HCV ribozymes directed against additional sites in the HCV minus strand. Both RPI motif I and motif II ribozymes are represented. HeLa cells in 96-well plates were infected with HCV-PV at a multiplicity of infection (MOI) of 0.1. Virus inoculum was then replaced with media containing 5% serum and ribozyme or control (200nM), as indicated, complexed to a cationic lipid. After 24 hours cells, were lysed 3 times by freeze/thaw and virus was quantified by plaque assay. Scrambled control (SAC) and ribozymes targeting different sites are indicated. Plaque forming units (pfu)/ml are shown as the mean of

triplicate samples + standard deviation (S.D.). Ribozymes used in this study are shown in **Table X.**

Figure 13 is a bar graph showing the dose response of a HCV minus strand site 205 directed anti-HCV ribozyme (RPI No. 15006, **Table X**). Plaque forming units (pfu)/ml are shown as the mean of triplicate samples + standard deviation (S.D.). Results are shown in plaque forming units (pfu)/ml vs. ribozyme concentration in nM.

Figure 14 is a graph showing the dose response of a HCV plus strand site 195 directed anti-HCV ribozyme (RPI No. 13919) when mixed with differing anti-HCV minus strand directed ribozymes (**Table X**). Results are shown in plaque forming units (pfu)/ml vs. ribozyme concentration in nM.

Ribozymes

Seven basic varieties of naturally-occurring enzymatic RNAs are known presently. In addition, several *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc. London,* B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing cleavage and ligation of phosphodiester linkages (Joyce, 1989, *Gene,* 82, 83-87; Beaudry *et al.,* 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.,* 1994, *TIBTECH* 12, 268; Bartel *et al.,*1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.,* 1995, *FASEB J.,* 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442; Santoro *et al.,* 1997, *Proc. Natl. Acad. Sci.,* 94, 4262; Tang *et al.,* 1997, *RNA* 3, 914; Nakamaye & Eckstein, 1994, *supra*; Long & Uhlenbeck, 1994, *supra*; Ishizaka *et al.,* 1995, *supra*; Vaish *et al.,* 1997, *Biochemistry* 36, 6495; all of these publications are incorporated by reference herein). Each can catalyze a series of reactions including the hydrolysis of phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. **Table I** summarizes some of the characteristics of some of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an

enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, since the concentration of ribozyme necessary to affect a therapeutic treatment is lower. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a ribozyme.

Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be targeted to virtually any RNA transcript, and efficient cleavage achieved *in vitro* (Zaug *et al.*, 324, *Nature* 429 1986 ; Uhlenbeck, 1987 *Nature* 328, 596; Kim et al., 84 *Proc. Natl. Acad. Sci. USA* 8788, 1987; Dreyfus, 1988, *Einstein Quart. J. Bio. Med.*, 6, 92; Haseloff and Gerlach, 334 *Nature* 585, 1988; Cech, 260 *JAMA* 3030, 1988; and Jefferies et al., 17 *Nucleic Acids Research* 1371, 1989; Chartrand *et al.*, 1995, *Nucleic Acids Research* 23, 4092; Santoro *et al.*, 1997, *PNAS* 94, 4262).

Because of their sequence-specificity, *trans*-cleaving ribozymes show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Ribozymes can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

Ribozymes that cleave the specified sites in HCV RNAs represent a novel therapeutic approach to infection by the hepatitis C virus. Applicant indicates that ribozymes are able to inhibit the activity of HCV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art will find that it is clear from the examples described that other ribozymes that cleave HCV RNAs may be readily designed and are within the invention.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper *et al.*, WO 93/23569; Sullivan *et al.*, WO 93/23057; Thompson *et al.*, WO 94/02595; Draper *et al.*, WO 95/04818; McSwiggen *et al.*, US Patent No. 5,525,468; and, are all hereby incorporated by reference herein in their totalities. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized and delivered as described therein.

The sequence of HCV RNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in **Tables IV-VIII and X** (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the tables as that site to be cleaved by the designated type of ribozyme. The nucleotide base position is noted in the tables as that site to be cleaved by the designated type of ribozyme.

Because HCV RNAs are highly homologous in certain regions, some ribozyme target sites are also homologous **(see Table IV and VIII)**. In this case, a single ribozyme will target different classes of HCV RNA. The advantage of one ribozyme that targets several classes of HCV RNA is clear, especially in cases where one or more of these RNAs may contribute to the disease state.

Hammerhead or hairpin ribozymes were designed that could bind and were individually analyzed by computer folding (Jaeger *et al.*, 1989 *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA. Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above.

Ribozyme Synthesis

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, antisense oligonucleotides, hammerhead or the Inozyme ribozymes) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

The method of synthesis used for normal RNA including certain enzymatic nucleic acid molecules follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684; and Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation

solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 µL of a solution of 1.5 mL N-methylpyrrolidinone, 750 µL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to r.t. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

Inactive hammerhead ribozymes were synthesized by substituting switching the order of G_5A_6 and substituting a U for A_{14} (numbering from Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252). Inactive ribozymes were also by synthesized by substituting a U for G5 and a U for A14. In some cases, the sequence of the substrate binding arms were randomized while the overall base composition was maintained.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format, all that is important is the ratio of chemicals used in the reaction.

Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51).

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204).

The nucleic acid molecules of the present invention are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and are re-suspended in water.

The sequences of the ribozymes that are chemically synthesized, useful in this study, are shown in **Tables IV to X**. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. The ribozyme sequences listed in **Tables IV to X** may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such

ribozymes with enzymatic activity are equivalent to the ribozymes described specifically in the tables.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases may increase their potency (see *e.g.,* Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken et al., 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; and Burgin *et al., supra;* all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules herein). All these publications are hereby incorporated by reference herein. Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H, nucleotide base modifications (for a review, see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.,* 1996, *Biochemistry* , 35, 14090). All of these publications are incorporated by reference herein. Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al. Nature*, 1990, *344*, 565-568; Pieken *et al. Science,* 1991, *253*, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.* , 1992, *17*, 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, US Patent No. 5,334,711 and Beigelman *et al.,* 1995, *J. Biol. Chem.,* 270, 25702; Beigelman *et al.,* International PCT publication No. WO 97/26270; Beigelman *et al.,* US Patent No. 5,716,824; Usman *et al.,* US patent No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.,* 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic acid Sciences),* 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina *et al.,*

1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without inhibiting catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, too many of these modifications may cause some toxicity. Therefore when designing nucleic acid molecules the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity resulting in increased efficacy and higher specificity of these molecules.

Nucleic acid molecules having chemical modifications which maintain or enhance activity are provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. Therapeutic nucleic acid molecules delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, nucleic acid molecules must be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

Use of these the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.*, multiple antisense or enzymatic nucleic acid molecules targeted to different genes, nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of molecules (including different motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules.

Therapeutic nucleic acid molecules (*e.g.*, enzymatic nucleic acid molecules) delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, these nucleic acid molecules must be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or *in vivo* where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of these properties is increased or not significantly (less that 10 fold) decreased *in vivo* compared to an all RNA ribozyme or all DNA enzyme.

In yet another preferred embodiment, nucleic acid catalysts having chemical modifications which maintain or enhance enzymatic activity is provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in a cell and/or *in vivo* even if activity over all is reduced 10-fold (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Such ribozymes herein are said to "maintain" the enzymatic activity of an all RNA ribozyme.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'- cap structure.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Wincott *et al.*, WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or may be present on both termini. In non-limiting examples: the 5'-cap is selected from the group comprising inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide

moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott *et al.*, International PCT publication No. WO 97/26270, incorporated by reference herein).

In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details, see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein). By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl

groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated p electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*. All these publications are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications

that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). All these publications are incorporated by reference herein. By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

In a preferred embodiment, the invention features modified ribozymes with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39. These references are hereby incorporated by reference herein.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, (for more details, see Wincott *et al.*, International PCT publication No. WO 97/26270).

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of β-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which may be modified or unmodified. Such modified groups are described, for example, in Eckstein et al., U.S. Patent 5,672,695 and Matulic-

Adamic et al., WO 98/28317, respectively, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid (*e.g.,* antisense and ribozyme) structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, *e.g.,* to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Use of these molecules will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes (including different ribozyme motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules. Therapies may be devised which include a mixture of ribozymes (including different ribozyme motifs), antisense and/or 2-5A chimera molecules to one or more targets to alleviate symptoms of a disease.

Administration of Ribozymes

Sullivan *et al.*, PCT WO 94/02595, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump, stent or other delivery devices such as Alzet® pumps, Medipad® devices. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan *et al.*, supra and Draper *et al.*, PCT WO93/23569, which have been incorporated by reference herein.

The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

The negatively charged polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a patient by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a lipid or liposome delivery mechanism, standard protocols for formulation can be followed. The compositions of the present invention may also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and the like.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, including salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or patient, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation to reach a target cell (*i.e.*, a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in* vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged polymers, *e.g.*, nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES).

A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach may provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as the HCV infected liver cells.

The invention also features the use of a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, **95**, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, **43**, 1005-1011). All these publications are incorporated by reference herein. Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, **267**, 1275-1276; Oku *et al.*,1995, *Biochim. Biophys. Acta*, **1238**, 86-90). All these references are incorporated by reference herein. The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, **42**, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392; all of these are incorporated by reference herein).

In addition other cationic molecules may also be utilized to deliver the molecules of the present invention. For example, ribozymes may be conjugated to glycosylated poly(L-lysine) which has been shown to enhance localization of antisense oligonucleotides into the liver (Nakazono *et al.*, 1996, *Hepatology* 23, 1297-1303; Nahato *et al.*, 1997, *Biochem Pharm.* 53, 887-895). Glycosylated poly (L-lysine) may be covalently attached to the enzymatic nucleic acid or be bound to enzymatic nucleic acid through electrostatic interaction.

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated

by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents may be provided. *Id.* at 1449. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents may be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the present invention may also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication may increase the beneficial effects while reducing the presence of side effects.

Alternatively, the enzymatic nucleic acid molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985 *Science* 229, 345; McGarry and Lindquist, 1986 *Proc. Natl. Acad. Sci.* USA 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992 *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992 *J. Virol*, 66, 1432-41; Weerasinghe *et al.*, 1991 *J. Virol*, 65, 5531-4; Ojwang *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89, 10802-6; Chen *et al.*, 1992 *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science* 247, 1222-1225; Thompson *et al.*, 1995 *Nucleic Acids Res.* 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45; all of these references are hereby incorporated in their totalities by reference herein). Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a ribozyme (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992 *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993 *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994 *J. Biol. Chem.* 269, 25856; all of these references are hereby incorporated in their totalities by reference herein).

In another aspect of the invention, enzymatic nucleic acid molecules that cleave target molecules are expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. The active ribozyme contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind target nucleic acid molecules such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features, an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention is disclosed. The nucleic acid sequence encoding the nucleic acid catalyst of the instant invention is operable linked in a manner which allows expression of that nucleic acid molecule.

In another aspect the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector may optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the

levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res..*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). All of these references are incorporated by reference herein. Several investigators have demonstrated that nucleic acid molecules, such as ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as ribozymes in cells (Thompson *et al., supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, US Patent No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736; all of these publications are incorporated by reference herein. The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as, adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review, see Couture and Stinchcomb, *1996, supra)*.

In yet another aspect, the invention features an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention, in a manner which allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In another preferred embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; d) a nucleic acid sequence encoding

at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

Interferons

Type I interferons (IFN) are a class of natural cytokines that includes a family of greater than 25 IFN-α (Pesta, 1986, *Methods Enzymol.* 119, 3-14) as well as IFN-β, and IFN-ω. Although evolutionarily derived from the same gene (Diaz *et al.*, 1994, *Genomics* 22, 540-552), there are many differences in the primary sequence of these molecules, implying an evolutionary divergence in biologic activity. All type I IFN share a common pattern of biologic effects that begin with binding of the IFN to the cell surface receptor (Pfeffer & Strulovici, 1992, Transmembrane secondary messengers for IFN-α/β. In: *Interferon. Principles and Medical Applications.*, S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tyring, eds. 151-160). Binding is followed by activation of tyrosine kinases, including the Janus tyrosine kinases and the STAT proteins, which leads to the production of several IFN-stimulated gene products (Johnson *et al.*, 1994, *Sci. Am.* 270, 68-75). The IFN-stimulated gene products are responsible for the pleotropic biologic effects of type I IFN, including antiviral, antiproliferative, and immunomodulatory effects, cytokine induction, and HLA class I and class II regulation (Pestka *et al.*, 1987, *Annu. Rev. Biochem* 56, 727). Examples of IFN-stimulated gene products include 2-5-oligoadenylate synthetase (2-5 OAS), β_2-microglobulin, neopterin, p68 kinases, and the

Mx protein (Chebath & Revel, 1992, The 2-5 A system: 2-5 A synthetase, isospecies and functions. In: *Interferon. Principles and Medical Applications.* S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Jr. Fleischmann, T.K. Jr Hughes, G.R. Kimpel, D.W. Niesel, G.J. Stanton, and S.K. Tyring, eds., pp. 225-236; Samuel, 1992, The RNA-dependent P1/eIF-2α protein kinase. In: *Interferon. Principles and Medical Applications*. S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.H. Stanton, and S.K. Tyring, eds. 237-250; Horisberger, 1992, MX protein: function and Mechanism of Action. In: *Interferon. Principles and Medical Applications.* S. Baron, D.H. Coopenhaver, F. Dianzani, W.R. Fleischmann Jr., T.K. Hughes Jr., G.R. Kimpel, D.W. Niesel, G.H. Stanton, and S.K. Tyring, eds. 215-224). Although all type I IFN have similar biologic effects, not all the activities are shared by each type I IFN, and, in many cases, the extent of activity varies quite substantially for each IFN subtype (Fish *et al*, 1989, *J. Interferon Res.* 9, 97-114; Ozes *et al.*, 1992, *J. Interferon Res.* 12, 55-59). More specifically, investigations into the properties of different subtypes of IFN-α and molecular hybrids of IFN-α have shown differences in pharmacological properties (Rubinstein, 1987, *J. Interferon Res.* 7, 545-551). These pharmacological differences may arise from as few as three amino acid residue changes (Lee *et al.*, 1982, *Cancer Res.* 42, 1312-1316).

Eighty-five to 166 amino acids are conserved in the known IFN-α subtypes. Excluding the IFN-α pseudogenes, there are approximately 25 known distinct IFN-α subtypes. Pairwise comparisons of these nonallelic subtypes show primary sequence differences ranging from 2% to 23%. In addition to the naturally occurring IFNs, a non-natural recombinant type I interferon known as consensus interferon (CIFN) has been synthesized as a therapeutic compound (Tong *et al.*, 1997, *Hepatology* 26, 747-754).

Interferon is currently in use for at least 12 different indications including infectious and autoimmune diseases and cancer (Borden, 1992, *N. Engl. J. Med.* 326, 1491-1492). For autoimmune diseases IFN has been utilized for treatment of rheumatoid arthritis, multiple sclerosis, and Crohn's disease. For treatment of cancer IFN has been used alone or in combination with a number of different compounds. Specific types of cancers for which IFN has been used include squamous cell carcinomas, melanomas, hypernephromas, hemangiomas, hairy cell leukemia, and Kaposi's sarcoma. In the treatment of infectious diseases, IFNs increase the phagocytic activity of macrophages and cytotoxicity of lymphocytes and inhibits the propagation of cellular pathogens. Specific indications for which IFN has been used as

treatment include: hepatitis B, human papillomavirus types 6 and 11 (i.e. genital warts) (Leventhal *et al.*, 1991, *N Engl J Med* 325, 613-617), chronic granulomatous disease, and hepatitis C virus.

Numerous well controlled clinical trials using IFN-alpha in the treatment of chronic HCV infection have demonstrated that treatment three times a week results in lowering of serum ALT values in approximately 50% (range 40% to 70%) of patients by the end of 6 months of therapy (Davis *et al.*, 1989, *New England Journal of Medicine* 321, 1501-1506; Marcellin *et al.*, 1991, *Hepatology* 13, 393-397; Tong *et al.*, 1997, Hepatology 26, 747-754; Tong *et al.*, *Hepatology* 26, 1640-1645). However, following cessation of interferon treatment, approximately 50% of the responding patients relapsed, resulting in a "durable" response rate as assessed by normalization of serum ALT concentrations of approximately 20 to 25%. In addition, studies that have examined six months of type 1 interferon therapy using changes in HCV RNA values as a clinical endpoint have demonstrated that up to 35% of patients will have a loss of HCV RNA by the end of therapy (Tong *et al.*, 1997, supra). However, as with the ALT endpoint, about 50% of the patients relapse six months following cessation of therapy resulting in a durable virologic response of only 12% (23). Studies that have examined 48 weeks of therapy have demonstrated that the sustained virological response is up to 25%.

Ribozymes in combination with IFN have the potential to improve the effectiveness of treatment of HCV or any of the other indications discussed above. Ribozymes targeting RNAs associated with diseases such as infectious diseases, autoimmune diseases, and cancer, can be used individually or in combination with other therapies such as IFN to achieve enhanced efficacy.

Examples

The following are non-limiting examples showing the selection, isolation, synthesis and activity of enzymatic nucleic acids of the instant invention.

The following examples demonstrate the selection of ribozymes that cleave HCV RNA. The methods described herein represent a scheme by which ribozymes may be derived that cleave other RNA targets required for HCV replication.

Example 1: Identification of Potential Ribozyme Cleavage Sites in HCV RNA

The sequence of HCV RNA was screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and contained potential hammerhead and/or hairpin ribozyme cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables IV-VIII, and X.**

Example 2: Selection of Ribozyme Cleavage Sites in HCV RNA

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in HCV RNA, 20 hammerhead sites were selected for analysis. Ribozyme target sites were chosen by analyzing genomic sequences of HCV (Input Sequence = HPCJTA (Acc#D11168 & D01171)) and prioritizing the sites on the basis of folding. Hammerhead ribozymes were designed that could bind each target (see Figure 1) and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem,* 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, **86**, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Selection of ribozyme candidates was initiated by scanning for all hammerhead cleavage sites in an HCV RNA sequence derived from a patient infected with HCV genotype 1b. The results of this sequence analysis are shown in **Table III**. As seen by **Table III**, 1300 hammerhead ribozyme sites were identified by this analysis. Next, in order to identify hammerhead ribozyme candidates that would cleave in the conserved regions of the HCV genome, a sequence alignment of approximately 50 HCV isolates from genotypes 1a, 1b, 2a, 2b, 2c, 3a, 3b, 4a, 5a, and 6 was completed. Within genotype sites were identified that are in areas having the greatest sequence identity between all isolates examined. This analysis reduced the hammerhead ribozyme candidates to about 23 (**Table III**).

Due to the high sequence variability of the HCV genome, selection of ribozymes for broad therapeutic applications should probably involve the conserved regions of the HCV genome. A list of the thirty-hammerhead ribozymes derived from the conserved regions (5'-Non-Coding Region (NCR), 5'- end of core protein coding region, and 3'- NCR) of the HCV genome is shown in **Table IV**. In general, ribozymes targeted to sites located in the 5' terminal

region of the HCV genome should block translation while ribozymes cleavage sites located in the 3' terminal region of the genome should block RNA replication.

Example 3: Chemical Synthesis and Purification of Ribozymes

Ribozymes of the hammerhead or hairpin motif were designed to anneal to various sites in the RNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described in Usman et al., (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe *et al.*, (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott *et al.*, *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Inactive hammerhead ribozymes were synthesized by substituting switching the order of G_5A_6 and substituting a U for A_{14} (numbering from Hertel *et al.*, 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes were synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes were modified to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review, see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Tables IV -X.**

Example 4: Ribozyme Cleavage of HCV RNA Target *in vitro*

Ribozymes targeted to the HCV are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example, using the following procedure. The target sequences and the nucleotide location within the HCV are given in **Table IV.**

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [$-^{32}P$] CTP, passed over a G 50 Sephadex® column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM $MgCl_2$) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.,* ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

Example 5: Ability of HCV Ribozymes to Cleave HCV RNA in patient serum.

Ribozymes targeting sites in HCV RNA were synthesized using modifications that confer nuclease resistance (Beigelman, 1995, *J. Biol. Chem.* 270, 25702). It has been well documented that serum from chronic hepatitis C patients contains on average 3 x 10^6 copies/ml of HCV RNA. To further select ribozyme product candidates, the 30 HCV specific ribozymes are characterized for HCV RNA cleavage activity utilizing HCV RNA isolated from the serum of genotype 1b HCV patients. The best candidates from the HCV genotype 1b screen will be screened against isolates from the wide range of HCV genotypes including 1a, 1b, 2a, 2b, 2c, 3a, 3b, 4a, 5a, and 6. Therefore, it is possible to select ribozyme candidates for further development based on their ability to broadly cleave HCV RNA from a diverse range of HCV genotypes and quasi-species.

Example 6: Ribozyme Cleavage of Conserved HCV RNA Target Sites *in vitro*

There are three regions of the genome that are highly conserved, both within a genotype and across different genotypes. These conserved sequences occur in the 5' and 3' non-coding

regions (NCRs) as well as the 5'-end of the Core Protein coding region. These regions are thought to be important for HCV RNA replication and translation. Thus, therapeutic agents that target these conserved HCV genomic regions may have a significant impact over a wide range of HCV genotypes. The presence of quasi-species, and the potential for infection with more than one genotype makes this a critical feature of an effective therapy. Moreover, it is unlikely that drug resistance will occur, since mutations that have been suggested to lead to drug resistance typically do not occur within these highly conserved regions. In order to target multiple genotypes and decrease the chance of developing drug resistance, Applicant has designed ribozymes that cleave in regions of identity within the conserved regions discussed above.

Sequence alignments were performed for the 5' NCR, the 5' end of the Core Protein coding region, and the 3' NCR. For the 5' NCR, 34 different isolates representing genotypes 1a, 1b, 2a, 2b, 2c, 3a, 3b, 4a, 4f, and 5a were aligned. The alignments included the sequences from nucleotide position 1 to nucleotide position 350 (18 nucleotides downstream of the initiator ATG codon), using the reported sequence "HPCK1S1" as the reference for numbering. For the Core Protein coding region, 44 different isolates representing genotypes 1a, 1b, 2a, 2b, 2c, 3a, 3b, 4a, 4c, 4f, 5a, and 6a were aligned. These alignments included 600 nucleotides, beginning 8 nucleotides upstream of the initiator ATG codon. As the reference for numbering, the reported sequence "HPCCOPR" was used, with the "C" eight nucleotides upstream of the initiator codon ATG designated as "1". For the 3' NCR region, 20 different isolates representing genotypes 1b, 2a, 2b, 3a, and 3b were aligned. These alignments included sequences in the 3' terminal 235 nucleotides of the genome, with the reported sequence "D85516" used as the reference for numbering, and the 235th nucleotide from the 3' end designated as "1".

During analysis of the alignments of each region, each sequence was compared to the respective reference sequence (identified above), and regions of identity across all isolates were determined. All potential ribozyme sites were identified in the reference sequence. The highest priority for choosing ribozyme sites was that the site should have 100% identity across all isolates aligned, at every position in both the cleavage site and binding arms. Ribozyme sites that met these criteria were chosen. In addition, two specific allowances were made as follows. 1) If a potential ribozyme site had 100% sequence identity at all except one or two nucleotide positions, then the actual nucleotide at that position was examined in the isolate(s) that differed. If that nucleotide was such that a ribozyme designed to allow "G:U wobble" base-

paring could function on all the isolates, then that site was chosen. 2) If a potential ribozyme site had 100% sequence identity at all except one or two nucleotide positions, then the genotype of the isolate which contained the differing nucleotide(s) was examined. If the genotype of the isolate that differed was of extremely rare prevalence, then that site was also chosen.

Ribozyme sites identified and referred to below use the following nomenclature: "region of the genome in which the site exists" followed by "nucleotide position 5' to the cleavage site" (according to the reference sequence and numbering described above). For example, a ribozyme cleavage site at nucleotide position 67 in the 5' NCR is designated "5-67", and a ribozyme cleavage site at position 48 in the core coding region is designated "c48".

A number of these ribozymes were screened in an *in vitro* HCV cleavage assay to select appropriate ribozyme candidates for cell culture studies. The ribozymes selected for screening targeted the 5' UTR region that is necessary for HCV translation. These sites are all conserved among the 8 major HCV genotypes and 18 subtypes, and have a high degree of homology in every HCV isolate that was used in the analysis described above. HCV RNA of four different genotypes (1b, 2a, 4, and 5) were isolated from human patients and the 5' HCV UTR and 5' core region were amplified using RT-PCR. Run-off transcripts of the 5' HCV UTR region (~750 nt transcripts) were prepared from the RT-PCR products, which contained a T7 promoter, using the T7 Megascript® transcription kit and the manufacturers protocol (Ambion, Inc.). Unincorporated nucleotides are removed by spin column filtration on Bio-Gel P-60 resin (Bio-Rad). The filtered transcript was 5' end labeled with ^{32}P using Polynucleotide Kinase (Boehringer/Mannheim) and 150μCi/μl Gamma-32P-ATP (NEN) using the enzyme manufacturer's protocol. The kinased transcript is spin purified again to remove unincorporated Gamma-32P-ATP and gel purified on 5% polyacrylamide gel.

Ribozymes targeting various sites from **Table IV** were selected and tested on the 5' HCV UTR transcript sequence to test the efficiency of RNA cleavage. 15 ribozymes were synthesized as previously described (Wincott *et al., supra*).

Assays were performed by pre-warming a 2X (2 μM) concentration of purified ribozyme in ribozyme cleavage buffer (50mM TRIS pH 7.5, 10mM $MgCl_2$, 10 units RNase Inhibitor (Boehringer/Mannheim), 10mM DTT, 0.5μg tRNA) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (17.46 pmole final

concentration) that was also pre-warmed in cleavage buffer. The assay was carried out for 24 hours at 37°C using a final concentration of 1 μM ribozyme, *i.e.*, ribozyme excess. The reaction was quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

Observed cleavage fragment sizes from the gels are correlated to predicted fragment sizes by comparison to the RNA marker. The optical density of expected cleavage fragments are determined from the phosphorimage plates and ranked from highest density, indicating the most cleavage product, to lowest of each genotype of HCV transcript tested. The top 3 cleaving ribozymes (out of 15 ribozymes tested) are given ranking values of 5, the next 3 highest densities are given ranking values of 4, *etc.* for every genotype tested. The ranking values for each ribozyme are averaged between the genotypes tested. Individual and average ribozyme ranking values are graphed and compared. The results (figure 2) demonstrate that many of these tested ribozymes are able to give high levels of cleavage regardless of genotype. In particular, ribozymes targeting site HCV.5-258, HCV.5-294, HCV.5-313 (Sakamoto *et al.*, *J Clinical Investigation* 1996 98(12):2720-2728), and HCV.5-318 (**Table IV**) appear to demonstrate a consistent pattern of RNA cleavage

Example 7:Inhibition of Luciferase Activity Using HCV Targeting Ribozymes in OST7 Cells

The capability of ribozymes to inhibit HCV RNA intracellularly was tested using a dual reporter system that utilizes both firefly and Renilla luciferase (figure 3). The ribozymes targeted to the 5' HCV UTR region, which when cleaved, would prevent the translation of the transcript into luciferase. OST-7 cells were plated at 12,500 cells per well in black walled 96 well plates (Packard) in medium DMEM containing 10% fetal bovine serum, 1% pen/strep, and 1% L-glutamine and incubated at 37°C overnight. A plasmid containing T7 promoter expressing 5' HCV UTR and firefly luciferase (T7C1-341 (Wang *et al.*, 1993, *J. of Virol.* 67, 3338-3344)) was mixed with a pRLSV40 Renilla control plasmid (Promega Corporation) followed by ribozyme, and cationic lipid to make a 5X concentration of the reagents (T7C1-

341 (4 μg/ml), pRLSV40 renilla luciferase control (6μg/ml), ribozyme (250 nM); transfection reagent (28.5μg/ml).

The complex mixture was incubated at 37°C for 20 minutes. The media was removed from the cells and 120 μl of Opti-mem media was added to the well followed by 30 μl of the 5X complex mixture. 150 μl of Opti-mem was added to the wells holding the untreated cells. The complex mixture was incubated on OST-7 cells for 4 hours, lysed with passive lysis buffer (Promega Corporation) and luminescent signals were quantified using the Dual Luciferase Assay Kit using the manufacturer's protocol (Promega Corporation). The ribozyme sequences used are given in **Table IV**. The ribozymes used were of the hammerhead motif. The hammerhead ribozymes were chemically modified such that the ribozyme consists of ribose residues at five positions (see for example Figure 7); position 4 has either 2'-C-allyl or 2'-amino modification; position 7 has either 2'-amino modification or 2-O-methyl modification; the remaining nucleotide positions contain 2'-*O*-methyl substitutions; four nucleotides at the 5' terminus contains phosphorothioate substitutions. Additionally, the 3' end of the ribozyme includes a 3'-3' linked inverted abasic moiety (abasic deoxyribose; iH). The data (figure 4) is given as a ratio between the firefly and Renilla luciferase fluorescence. All of the ribozymes targeting 5' HCV UTR were able to reduce firefly luciferase signal relative to renilla luciferase.

Example 9: Ribozyme Mediated Inhibition of Luciferase Activity Compared to its Inactive Control in OST-7 Cells

The dual reporter system described above was utilized to determine the level of reduction of luciferase activity mediated by a ribozyme compared to its inactive control. Ribozymes, having the chemical composition described in the previous example, to sites HCV 313 and 318 (**Table IV**) and their inactive controls were synthesized as above. The inactive control has the same nucleotide base composition as the active ribozyme but the nucleotide sequence has been scrambled. The protocols utilized for tissue culture and the luciferase assay was exactly as given in Example 8 except the ribozyme concentration in the 5X complex mixture was 1 mM (final concentration on the cells was 200 nM).

The results are given in figure 5. The ribozyme targeting HCV.5-318 was able to greatly reduce firefly luciferase activity compared to the untreated and inactive controls. The

ribozyme targeting HCV.5-313 was able to slightly reduce firefly luciferase activity compared to the inactive control.

Example 10: Ribozyme Inhibition of Viral Replication

During HCV infection, viral RNA is present as a potential target for ribozyme cleavage at several processes: uncoating, translation, RNA replication and packaging. Target RNA may be more or less accessible to ribozyme cleavage at any one of these steps. Although the association between the HCV initial ribosome entry site (IRES) and the translation apparatus is mimicked in the HCV 5'UTR/luciferase reporter system (Example 9), these other viral processes are not represented in the OST7 system. The resulting RNA/protein complexes associated with the target viral RNA are also absent. Moreover, these processes may be coupled in an HCV-infected cell which could further impact target RNA accessibility. Therefore, we tested whether ribozymes designed to cleave the HCV 5'UTR could effect a replicating viral system.

Recently, Lu and Wimmer characterized an HCV-poliovirus chimera in which the poliovirus IRES was replaced by the IRES from HCV (Lu & Wimmer, 1996, *Proc. Natl. Acad. Sci. USA.* 93, 1412-1417). Poliovirus (PV) is a positive strand RNA virus like HCV, but unlike HCV is non-enveloped and replicates efficiently in cell culture. The HCV-PV chimera expresses a stable, small plaque phenotype relative to wild type PV.

The following ribozymes were synthesized for the experiment (**Table VIII**): ribozyme targeting site 183 (3 5'-end phosphorothioate linkages), scrambled control to site 183, ribozyme to site 318 (3 5'-end phosphorothioate linkages), ribozyme targeting site 183 (4 5'-end phosphorothioate linkages), inactive ribozyme targeting site 183 (4 5'-end phosphorothioate linkages). HeLa cells were infected with the HCV-PV chimera for 30 minutes and immediately treated with ribozyme. HeLa cells were seeded in U-bottom 96-well plates at a density of 9000-10,000 cells/well and incubated at 37°C under 5% CO_2 for 24 h. Transfection of ribozyme (200 nM) was achieved by mixing of 10X ribozyme (2000 nM) and 10X of a cationic lipid (80 μg/ml) in DMEM (Gibco BRL) with 5% fetal bovine serum (FBS). Ribozyme/lipid complexes were allowed to incubate for 15 minutes at 37°C under 5% CO_2. Medium was aspirated from cells and replaced with 80 μls of DMEM (Gibco BRL) with 5% FBS serum, followed by the addition of 20 μls of 10X complexes. Cells were incubated with complexes for 24 hours at 37°C under 5% CO_2.

The yield of HCV-PV from treated cells (Fig. 6A) was quantified by plaque assay. The plaque assays were performed by diluting virus samples in serum-free DMEM (Gibco BRL) and applying 100 µl to HeLa cell monolayers (~80% confluent) in 6-well plates for 30 minutes. Infected monolayers were overlayed with 3 ml 1.2% agar (Sigma) and incubated at 37°C under 5% CO_2. Two - three days later the overlay was removed, monolayers were stained with 1.2% crystal violet, and plaque forming units were counted. The data is shown in figure 6A. Ribozymes to site 183 (after the full-length HCV clone was obtained site 183 now corresponds to site 195) inhibited HCV-PV replication by >80% ($P < 0.05$) compared to the scrambled control (Fig. 6A, first two bars). In addition, 3 or 4 phosphorothioate stabilization was equally effective ($P < 0.05$ vs. control for each) in inhibiting viral replication (compare 1st and 4th bar in Fig. 6A). Ribozymes to the 318 site also had a statistically significant ($P < 0.05$), effect on viral replication (compare 2nd and 3rd bar in Fig. 6A).

To confirm that a ribozyme cleavage mechanism was responsible for the inhibition of HCV-PV replication observed, HCV-PV infected cells were treated with ribozymes to site 183 that maintained binding arm sequences but contained a mutation in the catalytic core to attenuate cleavage activity (**Table I**). Viral replication in these cells was not inhibited compared to cells treated with the scrambled control ribozyme (Fig. 6A, 4th and 5th bar), indicating that ribozyme cleavage activity was required for the inhibition of HCV-PV replication observed. In addition, ribozymes targeting site 183 of the HCV 5'UTR had no effect on wild type PV replication (Fig. 6B). These data provide evidence that the ribozyme-mediated inhibition of HCV-PV replication was dependent upon the HCV 5' UTR and not a general inhibition of PV replication.

Ribozymes to site 183 were also tested for the ability to inhibit HCV-PV replication during a single infectious cycle in HeLa cells (Fig. 8). Cells treated with ribozyme to site 183 (7/4 format) produced significantly less virus than cells treated with the scrambled control (>80% inhibition at 8h post infection, $P < 0.001$).

Example 11: Shortening of Ribozyme lengths.

All the ribozymes described in example 10 above contained 7 nucleotides on each binding arms and contained a 4 base-paired stem II element (7/4 format). For pharmaceutical manufacture of a therapeutic ribozyme it is advantageous to minimize sequence length if possible. Thus ribozymes to site 183 were shortened by removing the outer most nucleotide

from each binding arm such that the ribozyme has six nucleotides in each binding arm and the stem II region is four base-paired long (6/4 format); removing one base-pair (2 nucleotides) in stem II resulting in a 3 base-paired stem II (7/3 format); or removing one nucleotide from each binding arm and shortening the stem II by one base-pair (6/3 format). (See Figure 7 for a schematic representation of each of these ribozymes). Ribozymes in all tested formats gave significant inhibition of viral replication (Fig. 8) with the 7/4, 7/3 and 6/3 formats being almost identical at the 8h timepoint ($P < 0.001$ across time course for all formats). The shortest ribozyme tested (6/3 format) was slightly more efficacious (>90% inhibition, $P < 0.001$) than the 7/4 ribozyme (~80% inhibition, $P < 0.001$). The 6/3 ribozyme may have a greater ability to access site 183 in the HCV-PV chimera.

Example 12: Combination Therapy of HCV Ribozymes and Interferon

HeLa cells (10,000 cells per well) were pre-treated with 12.5 Units/ml of Interferon alpha in complete media (DMEM + 5% FBS) or pre-treated with complete media alone for 4 hours and then infected with HCV-PV at an MOI = 0.1 for 30 minutes. The viral inoculum was then removed and 200 nM ribozyme targeted to HCV site 183 (Rz) or binding attenuated control, which has mutations in the catalytic core of the ribozyme that severely attenuates the activity of the ribozyme, (BAC) was delivered using cationic lipid in complete media for 24 hours. After 24 hours, the cells were lysed three times by freeze/thaw to release virus and virus was quantified by plaque assay. Viral yield is shown as mean plaque forming units per ml (pfu/ml) + SEM. The data is shown in figure 10.

Pre-treatment with interferon (IFN) reduces the viral yield by $\sim 10^{-1}$ in control treated cells (BAC+IFN versus BAC). Ribozyme treated cells produce 2×10^{-1} less virus than control-treated cells (Rz versus BAC). The combination of Rz and IFN treatment results in a synergistic 4×10^{-2} reduction in viral yield (Rz+IFN versus BAC). An additive effect would result in only a 3×10^{-1} reduction ($1 \times 10^{-1} + 2 \times 10^{-1}$).

Example 13: Inhibition of Hepatitis C virus Using various Ribozyme Motifs

A number of varying ribozyme motifs (RPI motifs I-III; Figure 9), were tested for their ability to inhibit HCV propagation in tissue culture. An example of RPI motif I (G-cleaver) is described in Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120, while an example of RPI motif II (Inozyme) is described in Ludwig & Sproat, International PCT Publication No. WO 98/58058). RPI motif III is a new ribozyme motif which applicant has recently developed and an example of this motif was tested herein.

OST7 cells were maintained in Dulbecco's modified Eagle's medium (GIBCO BRL) supplemented with 10% fetal calf serum, L-glutamine (2mM) and penicillin/streptomycin. For transfections, OST7 cells were seeded in black-walled 96-well plates (Packard Instruments) at a density of 12,500 cells/well and incubated at 37°C under 5% CO_2 for 24 hours. Co-transfection of target reporter HCVT7C (0.8 g/ml), control reporter pRLSV40, (1.2 μg/ml) and ribozyme, 50-200 nM was achieved by the following method: a 5X mixture of HCVT7C (4 μg/ml), pRLSV40 (6 μg/ml), ribozyme (250–1000 nM) and cationic lipid (28.5 μg/ml) was made in 150 μls of OPTI-MEM (GIBCO BRL) minus serum. Reporter/ribozyme/lipid complexes were allowed to form for 20 minutes at 37°C under 5% CO_2. Medium was aspirated from OST7 cells and replaced with 120 μls of OPTI-MEM (GIBCO BRL) minus serum, immediately followed by the addition of 30 μls of 5X reporter/ribozyme/lipid complexes. Cells were incubated with complexes for 4 hours at 37°C under 5% CO_2 . Luciferase assay was performed as described in example 7. The data is summarized in **Table IX**, with each motif's results listed along with its control. All of the ribozyme motifs were able to reduce the amount of HCV produced by the cells compared to the ribozymes not targeted to any HCV (irrelevant controls).

Example 14: General Protocol for Virus Infection and Ribozyme Delivery

HeLa cells were seeded in 96-well plates at a density of 9000-10,000 cells/well and incubated at 37°C under 5% CO_2 for 24 h. Cells were infected with HCV-PV at an MOI = 0.1 for 30 min. Transfection of ribozyme or control oligonucleotides (200 nM final) was achieved by mixing of 5X ribozyme or control oligonucleotides (1000 nM) and 5X cationic lipid (40 μg/ml at 5X, 800 ng/well final) in DMEM with 5% fetal bovine serum (FBS) in U-bottom 96-well plates. Ribozyme/lipid complexes were allowed to incubate for 15 min at 37°C under 5%

CO_2. Medium was aspirated from cells and replaced with 80 µl of DMEM with 5% FBS serum, followed by the addition of 20 µl of 5X complexes. Cells were incubated with complexes for 24 h at 37°C under 5% CO_2. After 24 h cells were lysed by three freeze/thaw cycles to release virus and virus was quantified by plaque assay.

Example 15: General Protocol for HCV plaque assay

Virus samples were diluted in serum-free DMEM and 100 µl applied to HeLa cell monolayers (~80% confluent) in 6-well plates for 30 min. Infected monolayers were overlayed with 3 ml 1.2% agar (Sigma, St. Louis, MO) and incubated at 37°C under 5% CO_2. When plaques were visible (after two to three days) the overlay was removed, monolayers were stained with 1.2% crystal violet, and plaque forming units were counted.

Example 16: Inhibition of Hepatitis C virus Using other Ribozyme Directed Against the HCV minus strand

HeLa cells in 96-well plates were infected with a chimeric Hepatitis C-Poliovirus (HCV-PV) at a multiplicity of infection (MOI) of 0.1. Virus inoculum was then replaced with media containing 5% serum and 200 nM ribozyme (**Table X**) or scrambled attenuated control (SAC), as indicated, complexed to cationic lipid. After 24 h cells were lysed 3 times by freeze/thaw and virus was quantified by plaque assay. Results are summarized in **Figures 11 and 12.** Plaque forming units (pfu)/ml are shown as the mean of triplicate samples + S.D.

Example 17: Dose Response of Ribozyme Directed Against the HCV minus strand

Cells were infected and treated with ribozyme as described in Example 16 except that various amounts (as indicated) of anti-HCV ribozyme RPI.15006 was mixed with a control oligonucleotide (SAC) to maintain a constant 200 nM total dose of nucleic acid for delivery. **Figure 13** shows the results of this study that demonstrates an effective dose response in cells to treatment with a ribozyme directed against the HCV minus strand.

Example 18: Dose Response of Ribozyme Directed Against the HCV plus strand combined with Ribozymes targeting the HCV minus strand

Cells were infected and treated with ribozyme as described in Example 16 except that various amounts (as indicated) of anti-HCV ribozyme RPI.13919, targeting the plus strand, was mixed with ribozymes targeting the minus strand, as noted, to maintain a constant 200 nM total dose of nucleic acid for delivery. **Figure 14** shows the results of this study that demonstrates an effective dose response in cells to treatment with a ribozyme (RPI 13919) directed against the HCV plus strand combined with a ribozyme targeting the HCV minus strand (RPI 14975).

Example 19: Inhibition of HCV *in vivo*

Ribozyme directed reduction of HCV *in vivo* was examined in a mouse model, generally described in Vierling, International PCT Publication No. WO 99/16307, using HCV RNA as an endpoint. The study compared mice treated with ribozymes compared to scrambled-attenuated-core ribozymes (SAC) and saline controls. Active ribozyme and SAC were dosed from day 5 through 20 post-transplant. Various modes of analysis were used including ANOVA of raw quantitative HCV RNA, Dunnett's of raw quantitative HCV RNA, ANOVA of $\log_{10}$ quantitative HCV RNA, Dunnett's of $\log_{10}$ quantitative HCV RNA, and Chi Square of qualitative results (HCV RNA +/-). Treatment with active ribozyme (RPI 13918), resulted in significant reduction of HCV RNA at 12 and 16 days using quantitative analysis ($p<0.05$ by Dunnett's using the $\log_{10}$ transformed HCV RNA results for all observations). The use of qualitative assessment, by converting the quantitative data into positive or negative results, confirmed with same trend. This study suggests that treatment with active anti-HCV ribozymes results in a significant reduction in HCV RNA in a trimeric mouse model.

CELL CULTURE ASSAYS

Although there have been reports of replication of HCV in cell culture (see below), these systems are difficult to replicate and have proven unreliable. Therefore, as was the case for

development of other anti-HCV therapeutics such as interferon and ribavirin, after demonstration of safety in animal studies applicant can proceed directly into a clinical feasibility study.

Several recent reports have documented *in vitro* growth of HCV in human cell lines (Mizutani *et al., Biochem Biophys Res Commun* 1996 227(3):822-826; Tagawa *et al., Journal of Gasteroenterology and Hepatology* 1995 10(5):523-527; Cribier *et al., Journal of General Virology* 76(10):2485-2491; Seipp *et al., Journal of General Virology* 1997 78(10)2467-2478; Iacovacci *et al., Research Virology* 1997 148(2):147-151; Iocavacci *et al., Hepatology* 1997 26(5) 1328-1337; Ito *et al., Journal of General Virology* 1996 77(5):1043-1054; Nakajima *et al., Journal of Virology* 1996 70(5):3325-3329; Mizutani *et al., Journal of Virology* 1996 70(10):7219-7223; Valli *et al., Res Virol* 1995 146(4): 285-288; Kato *et al., Biochem Biophys Res Comm* 1995 206(3):863-869). Replication of HCV has been demonstrated in both T and B cell lines as well as cell lines derived from human hepatocytes. Demonstration of replication was documented using either RT-PCR based assays or the b-DNA assay. It is important to note that the most recent publications regarding HCV cell cultures document replication for up to 6-months.

In addition to cell lines that can be infected with HCV, several groups have reported the successful transformation of cell lines with cDNA clones of full-length or partial HCV genomes (Harada *et al.,* Journal of General Virology 1995 76(5)1215-1221; Haramatsu *et al., Journal of Viral Hepatitis* 1997 4S(1):61-67; Dash *et al., American Journal of Pathology* 1997 151(2):363-373; Mizuno *et al., Gasteroenterology* 1995 109(6):1933-40; Yoo *et al., Journal Of Virology* 1995 69(1):32-38).

ANIMAL MODELS

The best characterized animal system for HCV infection is the chimpanzee. Moreover, the chronic hepatitis that results from HCV infection in chimpanzees and humans is very similar. Although clinically relevant, the chimpanzee model suffers from several practical impediments that make use of this model difficult. These include; high cost, long incubation requirements and lack of sufficient quantities of animals. Due to these factors, a number of

groups have attempted to develop rodent models of chronic hepatitis C infection. While direct infection has not been possible several groups have reported on the stable transfection of either portions or entire HCV genomes into rodents (Yamamoto *et al., Hepatology* 1995 22(3): 847-855; Galun *et al., Journal of Infectious Disease* 1995 172(1):25-30; Koike *et al., Journal of General Virology* 1995 76(12)3031-3038; Pasquinelli *et al., Hepatology* 1997 25(3): 719-727; Hayashi *et al., Princess Takamatsu Symp* 1995 25:1430149; Mariya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *Journal of General Virology* 1997 78(7) 1527-1531; Takehara *et al., Hepatology* 1995 21(3):746-751; Kawamura *et al., Hepatology* 1997 25(4): 1014-1021). In addition, transplantation of HCV infected human liver into immunocompromised mice results in prolonged detection of HCV RNA in the animal's blood.

Vierling, International PCT Publication No. WO 99/16307, describes a method for expressing hepatitis C virus in an *in vivo* animal model. Viable, HCV infected human hepatocytes are transplanted into a liver parenchyma of a scid/scid mouse host. The scid/scid mouse host is then maintained in a viable state, whereby viable, morphologically intact human hepatocytes persist in the donor tissue and hepatitis C virus is replicated in the persisting human hepatocytes. This model provides an effective means for the study of HCV inhibition by ribozymes *in vivo.*

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of *HCV* RNA in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses

of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with HCV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme can be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA can be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates can also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis can involve two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products can be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, HCV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Additional Uses

Potential usefulness of sequence-specific enzymatic nucleic acid molecules of the instant invention might have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans *et al.*, 1975 *Ann. Rev. Biochem.* 44:273). For example, the pattern of restriction fragments could be used to establish sequence relationships between two related RNAs, and large RNAs could be specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the enzymatic nucleic acid molecule is ideal for cleavage of RNAs of unknown sequence. Applicant describes the use of nucleic acid molecules to down-regulate gene expression of

target genes in bacterial, microbial, fungal, viral, and eukaryotic systems including plant, or mammalian cells.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Other embodiments are within the following claims.

TABLE I

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-OH of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintainance of the active structure.
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [i,ii].
- Complete kinetic framework established for one ribozyme [iii,iv,v,vi].
- Studies of ribozyme folding and substrate docking underway [vii,viii,ix].
- Chemical modification investigation of important residues well established [x,xi].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the Tetrahymena group I intron has been used to repair a "defective" -galactosidase message by the ligation of new -galactosidase sequences onto the defective message [xii].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [xiii].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3'-OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [xiv,xv]
- Important phosphate and 2' OH contacts recently identified [xvi,xvii]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [xviii,xix].

- Sequence requirements not fully determined.
- Reaction mechanism: 2'-OH of an internal adenosine generates cleavage products with 3'-OH and a "lariat" RNA containing a 3'-5' and a 2'-5' branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [xx,xxi] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [xxii].
- Important 2' OH contacts beginning to be identified [xxiii]
- Kinetic framework under development [xxiv]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [xxv].
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme

(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures [xxvi, xxvii]
- Minimal ligation activity demonstrated (for engineering through *in vitro* selection) [xxviii]
- Complete kinetic framework established for two or more ribozymes [xxix].
- Chemical modification investigation of important residues well established [xxx].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [xxxi, xxxii, xxxiii, xxxiv]

- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through *in vitro* selection [xxxv]
- Complete kinetic framework established for one ribozyme [xxxvi].
- Chemical modification investigation of important residues begun [xxxvii, xxxviii].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [xxxix].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [xl].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [xli]

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Table II: 2.5 μmol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 μL	2.5
S-Ethyl Tetrazole	23.8	238 μL	2.5
Acetic Anhydride	100	233 μL	5 sec
N-Methyl Imidazole	186	233 μL	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table III: Ribozyme Selection Characteristics

Characteristic	Number
HCV Genome Length	9436 kb
All Hammerhead Cleavage Sites *	1300
Conserved Region Hammerhead Cleavage Sites **	23

* HCV Genotype 1b was the prototype strain

** . Based on sequence alignments from HCV genotype 1a, 1b, 1c, 2a, 2b, 2c 3a, 3b, 4a, 4c, 4f, 5a, and 6a

Table IV: Hammerhead Ribozymes Derived from Conserved Regions of the HCV Genome

Name	Substrate	Ribozyme Sequence
5' NCR		
HCV.5-50	CUACUGU C UUCACGC	GCGUGAA CUGAUGAGGCCGUUAGGCCGAA ACAGUAG
HCV.5-67	AAAGCGU C UAGCCAU	AUGGCUA CUGAUGAGGCCGUUAGGCCGAA ACGCUUU
HCV.5-69	AGCGUCU A GCCAUGG	CCAUGGC CUGAUGAGGCCGUUAGGCCGAA AGACGCU
HCV.5-92	UGAGUGU C GUGCAGC	GCUGCAC CUGAUGAGGCCGUUAGGCCGAA ACACUCA
HCV.5-130	GAGCCAU A GUGGUCU	AGACCAC CUGAUGAGGCCGUUAGGCCGAA AUGGCUC
HCV.5-136	UAGUGGU C UGCGGAA	UUCCGCA CUGAUGAGGCCGUUAGGCCGAA ACCACUA
HCV.5-153	GGUGAGU A CACCGGA	UCCGGUG CUGAUGAGGCCGUUAGGCCGAA ACUCACC
HCV.5-180	ACCGGGU C CUUUCUU	AAGAAAG CUGAUGAGGCCGUUAGGCCGAA ACCCGGU
HCV.5-183	GGGUCCU U UCUUGGA	UCCAAGA CUGAUGAGGCCGUUAGGCCGAA AGGACCC
HCV.5-184	GGUCCUU U CUUGGAU	AUCCAAG CUGAUGAGGCCGUUAGGCCGAA AAGGACC
HCV.5-258	GUUGGGU C GCGAAAG	CUUUCGC CUGAUGAGGCCGUUAGGCCGAA ACCCAAC
HCV.5-270	AAGGCCU U GUGGUAC	GUACCAC CUGAUGAGGCCGUUAGGCCGAA AGGCCUU
HCV.5-294	GGGUGCU U GCGAGUG	CACUCGC CUGAUGAGGCCGUUAGGCCGAA AGCACCC
HCV.5-313	GGGAGGU C UCGUAGA	UCUACGA CUGAUGAGGCCGUUAGGCCGAA ACCUCCC
HCV.5-315	GAGGUCU C GUAGACC	GGUCUAC CUGAUGAGGCCGUUAGGCCGAA AGACCUC
HCV.5-318	GUCUCGU A GACCGUG	CACGGUC CUGAUGAGGCCGUUAGGCCGAA ACGAGAC
Core Region		
HCV.C-30	UAAACCU C AAAGAAA	UUUCUUU CUGAUGAGGCCGUUAGGCCGAA AGGUUUA
HCV.C-48	CAAACGU A ACACCAA	UUGGUGU CUGAUGAGGCCGUUAGGCCGAA ACGUUUG
HCV.C-60	CAACCGU C GCCCACA	UGUGGGC CUGAUGAGGCCGUUAGGCCGAA ACGGUUG
HCV.C-175	GAGCGGU C ACAACCU	AGGUUGU CUGAUGAGGCCGUUAGGCCGAA ACCGCUC
HCV.C-374	GUAAGGU C AUCGAUA	UAUCGAU CUGAUGAGGCCGUUAGGCCGAA ACCUUAC
3' NCR		
HCV.3-118	UUUUUUU U UUUUUUU	AAAAAAA CUGAUGAGGCCGUUAGGCCGAA AAAAAAA
HCV.3-145	GGUGGCU C CAUCUUA	UAAGAUG CUGAUGAGGCCGUUAGGCCGAA AGCCACC
HCV.3-149	GCUCCAU C UUAGCCC	GGGCUAA CUGAUGAGGCCGUUAGGCCGAA AUGGAGC
HCV.3-151	UCCAUCU U AGCCCUA	UAGGGCU CUGAUGAGGCCGUUAGGCCGAA AGAUGGA
HCV.3-152	CCAUCUU A GCCCUAG	CUAGGGC CUGAUGAGGCCGUUAGGCCGAA AAGAUGG
HCV.3-158	UAGCCCU A GUCACGG	CCGUGAC CUGAUGAGGCCGUUAGGCCGAA AGGGCUA
HCV.3-161	CCCUAGU C ACGGCUA	UAGCCGU CUGAUGAGGCCGUUAGGCCGAA ACUAGGG
HCV.3-168	CACGGCU A GCUGUGA	UCACAGC CUGAUGAGGCCGUUAGGCCGAA AGCCGUG
HCV.3-181	GAAAGGU C CGUGAGC	GCUCACG CUGAUGAGGCCGUUAGGCCGAA ACCUUUC

Table V: HCV Hammerhead Ribozyme and Target Sequence

No.	Name	Nt. Pos.	Hammerhead Ribozyme	Substrate
1	HCV-27	27	UAUGGUG CUGAUGAG X CGAA AGUGUCG	CGACACU C CACCAUA
2	HCV-114	114	GGUCCUG CUGAUGAG X CGAA AGGCUGC	GCAGCCU C CAGGACC
3	HCV-128	128	CUCCCGG CUGAUGAG X CGAA AGGGGGG	CCCCCCU C CCGGGAG
4	HCV-148	148	UUCCGCA CUGAUGAG X CGAA ACCACUA	UAGUGGU C UGCGGAA
5	HCV-165	165	UCCGGUG CUGAUGAG X CGAA ACUCACC	GGUGAGU A CACCGGA
6	HCV-175	175	UCCUGGC CUGAUGAG X CGAA AUUCCGG	CCGGAAU U GCCAGGA
7	HCV-199	199	UUGAUCC CUGAUGAG X CGAA AGAAAGG	CCUUUCU U GGAUCAA
8	HCV-213	213	AGGCAUU CUGAUGAG X CGAA AGCGGGU	ACCCGCU C AAUGCCU
9	HCV-252	252	ACUCGGC CUGAUGAG X CGAA AGCAGUC	GACUGCU A GCCGAGU
10	HCV-260	260	CCAACAC CUGAUGAG X CGAA ACUCGGC	GCCGAGU A GUGUUGG
11	HCV-265	265	GCGACCC CUGAUGAG X CGAA ACACUAC	GUAGUGU U GGGUCGC
12	HCV-270	270	CUUUCGC CUGAUGAG X CGAA ACCCAAC	GUUGGGU C GCGAAAG
13	HCV-288	288	CAGGCAG CUGAUGAG X CGAA ACCACAA	UUGUGGU A CUGCCUG
14	HCV-298	298	AGCACCC CUGAUGAG X CGAA AUCAGGC	GCCUGAU A GGGUGCU
15	HCV-306	306	CACUCGC CUGAUGAG X CGAA AGCACCC	GGGUGCU U GCGAGUG
16	HCV-325	325	UCUACGA CUGAUGAG X CGAA ACCUCCC	GGGAGGU C UCGUAGA
17	HCV-327	327	GGUCUAC CUGAUGAG X CGAA AGACCUC	GAGGUCU C GUAGACC
18	HCV-330	330	CACGGUC CUGAUGAG X CGAA ACGAGAC	GUCUCGU A GACCGUG
19	HCV-407	407	GGAACUU CUGAUGAG X CGAA ACGUCCU	AGGACGU C AAGUUCC
20	HCV-412	412	GCCCGGG CUGAUGAG X CGAA ACUUGAC	GUCAAGU U CCCGGGC
21	HCV-413	413	CGCCCGG CUGAUGAG X CGAA AACUUGA	UCAAGUU C CCGGGCG
22	HCV-426	426	ACGAUCU CUGAUGAG X CGAA ACCACCG	CGGUGGU C AGAUCGU
23	HCV-472	472	CACACCC CUGAUGAG X CGAA ACGUGGG	CCCACGU U GGGUGUG
24	HCV-489	489	GUCUUCC CUGAUGAG X CGAA AGUCGCG	CGCGACU A GGAAGAC
25	HCV-498	498	CGUUCGG CUGAUGAG X CGAA AGUCUUC	GAAGACU U CCGAACG
26	HCV-499	499	CCGUUCG CUGAUGAG X CGAA AAGUCUU	AAGACUU C CGAACGG
27	HCV-508	508	AGGUUGC CUGAUGAG X CGAA ACCGUUC	GAACGGU C GCAACCU
28	HCV-534	534	UUGGGGA CUGAUGAG X CGAA AGGUUGU	ACAACCU A UCCCCAA
29	HCV-536	536	CCUUGGG CUGAUGAG X CGAA AUAGGUU	AACCUAU C CCCAAGG
30	HCV-546	546	GGUCGGC CUGAUGAG X CGAA AGCCUUG	CAAGGCU C GCCGACC
31	HCV-561	561	CAGGCCC CUGAUGAG X CGAA ACCCUCG	CGAGGGU A GGGCCUG
32	HCV-573	573	CCAGGCU CUGAUGAG X CGAA AGCCCAG	CUGGGCU C AGCCUGG
33	HCV-583	583	CCAAGGG CUGAUGAG X CGAA ACCCAGG	CCUGGGU A CCCUUGG
34	HCV-588	588	AGGGGCC CUGAUGAG X CGAA AGGGUAC	GUACCCU U GGCCCCU
35	HCV-596	596	UGCCAUA CUGAUGAG X CGAA AGGGGCC	GGCCCCU C UAUGGCA
36	HCV-598	598	AUUGCCA CUGAUGAG X CGAA AGAGGGG	CCCCUCU A UGGCAAU
37	HCV-632	632	GUGACAG CUGAUGAG X CGAA AGCCAUC	GAUGGCU C CUGUCAC
38	HCV-637	637	GCGGGGU CUGAUGAG X CGAA ACAGGAG	CUCCUGU C ACCCCGC
39	HCV-649	649	AGGCCGG CUGAUGAG X CGAA AGCCGCG	CGCGGCU C CCGGCCU
40	HCV-657	657	CCCCAAC CUGAUGAG X CGAA AGGCCGG	CCGGCCU A GUUGGGG
41	HCV-660	660	GGGCCCC CUGAUGAG X CGAA ACUAGGC	GCCUAGU U GGGGCCC
42	HCV-696	696	UUACCCA CUGAUGAG X CGAA AUUGCGC	GCGCAAU C UGGGUAA
43	HCV-707	707	UAUCGAU CUGAUGAG X CGAA ACCUUAC	GUAAGGU C AUCGAUA

44	HCV-710	710	GGGUAUC CUGAUGAG X CGAA AUGACCU	AGGUCAU C GAUACCC
45	HCV-714	714	GUGAGGG CUGAUGAG X CGAA AUCGAUG	CAUCGAU A CCCUCAC
46	HCV-730	730	GUCGGCG CUGAUGAG X CGAA AGCCGCA	UGCGGCU U CGCCGAC
47	HCV-731	731	GGUCGGC CUGAUGAG X CGAA AAGCCGC	GCGGCUU C GCCGACC
48	HCV-748	748	CGGAAUG CUGAUGAG X CGAA ACCCCAU	AUGGGGU A CAUUCCG
49	HCV-752	752	CGAGCGG CUGAUGAG X CGAA AUGUACC	GGUACAU U CCGCUCG
50	HCV-753	753	ACGAGCG CUGAUGAG X CGAA AAUGUAC	GUACAUU C CGCUCGU
51	HCV-758	758	CGCCGAC CUGAUGAG X CGAA AGCGGAA	UUCCGCU C GUCGGCG
52	HCV-761	761	GGGCGCC CUGAUGAG X CGAA ACGAGCG	CGCUCGU C GGCGCCC
53	HCV-773	773	CGCCCCC CUGAUGAG X CGAA AGGGGGG	CCCCCCU A GGGGGCG
54	HCV-806	806	GAACCCG CUGAUGAG X CGAA ACACCAU	AUGGUGU C CGGGUUC
55	HCV-812	812	CCUCCAG CUGAUGAG X CGAA ACCCGGA	UCCGGGU U CUGGAGG
56	HCV-813	813	UCCUCCA CUGAUGAG X CGAA AACCCGG	CCGGGUU C UGGAGGA
57	HCV-832	832	UGUUGCG CUGAUGAG X CGAA AGUUCAC	GUGAACU A CGCAACA
58	HCV-847	847	ACCGGGC CUGAUGAG X CGAA AGUUCCC	GGGAACU U GCCCGGU
59	HCV-855	855	AAAGAGC CUGAUGAG X CGAA ACCGGGC	GCCCGGU U GCUCUUU
60	HCV-859	859	AGAGAAA CUGAUGAG X CGAA AGCAACC	GGUUGCU C UUUCUCU
61	HCV-982	982	UGCCUCA CUGAUGAG X CGAA ACACAAU	AUUGUGU A UGAGGCA
62	HCV-1001	1001	UAUGCAU CUGAUGAG X CGAA AUCAUGC	GCAUGAU C AUGCAUA
63	HCV-1022	1022	CGCAGGG CUGAUGAG X CGAA ACGCACC	GGUGCGU A CCCUGCG
64	HCV-1031	1031	UCUCCCG CUGAUGAG X CGAA ACGCAGG	CCUGCGU U CGGGAGA
65	HCV-1032	1032	UUCUCCC CUGAUGAG X CGAA AACGCAG	CUGCGUU C GGGAGAA
66	HCV-1048	1048	ACAACGG CUGAUGAG X CGAA AGGCGUU	AACGCCU C CCGUUGU
67	HCV-1053	1053	ACCCAAC CUGAUGAG X CGAA ACGGGAG	CUCCCGU U GUUGGGU
68	HCV-1056	1056	GCUACCC CUGAUGAG X CGAA ACAACGG	CCGUUGU U GGGUAGC
69	HCV-1061	1061	UGAGCGC CUGAUGAG X CGAA ACCCAAC	GUUGGGU A GCGCUCA
70	HCV-1127	1127	GCAAGUC CUGAUGAG X CGAA ACGUGGC	GCCACGU C GACUUGC
71	HCV-1132	1132	AACGAGC CUGAUGAG X CGAA AGUCGAC	GUCGACU U GCUCGUU
72	HCV-1136	1136	CCCCAAC CUGAUGAG X CGAA AGCAAGU	ACUUGCU C GUUGGGG
73	HCV-1139	1139	CCGCCCC CUGAUGAG X CGAA ACGAGCA	UGCUCGU U GGGGCGG
74	HCV-1153	1153	GGAACAG CUGAUGAG X CGAA AAGCGGC	GCCGCUU U CUGUUCC
75	HCV-1154	1154	CGGAACA CUGAUGAG X CGAA AAAGCGG	CCGCUUU C UGUUCCG
76	HCV-1158	1158	AUGGCGG CUGAUGAG X CGAA ACAGAAA	UUUCUGU U CCGCCAU
77	HCV-1159	1159	CAUGGCG CUGAUGAG X CGAA AACAGAA	UUCUGUU C CGCCAUG
78	HCV-1168	1168	CCCCACG CUGAUGAG X CGAA ACAUGGC	GCCAUGU A CGUGGGG
79	HCV-1189	1189	GAAAACG CUGAUGAG X CGAA AUCCGCA	UGCGGAU C CGUUUUC
80	HCV-1193	1193	CGAGGAA CUGAUGAG X CGAA ACGGAUC	GAUCCGU U UUCCUCG
81	HCV-1194	1194	ACGAGGA CUGAUGAG X CGAA AACGGAU	AUCCGUU U UCCUCGU
82	HCV-1195	1195	GACGAGG CUGAUGAG X CGAA AAACGGA	UCCGUUU U CCUCGUC
83	HCV-1196	1196	AGACGAG CUGAUGAG X CGAA AAAACGG	CCGUUUU C CUCGUCU
84	HCV-1280	1280	GACCUGA CUGAUGAG X CGAA ACAUGGC	GCCAUGU A UCAGGUC
85	HCV-1282	1282	GUGACCU CUGAUGAG X CGAA AUACAUG	CAUGUAU C AGGUCAC
86	HCV-1287	1287	AUGCGGU CUGAUGAG X CGAA ACCUGAU	AUCAGGU C ACCGCAU
87	HCV-1373	1373	UAUCCAC CUGAUGAG X CGAA ACAGCUU	AAGCUGU C GUGGAUA
88	HCV-1380	1380	GCCACCA CUGAUGAG X CGAA AUCCACG	CGUGGAU A UGGUGGC
89	HCV-1406	1406	CCGCUAG CUGAUGAG X CGAA ACUCCCC	GGGGAGU C CUAGCGG
90	HCV-1409	1409	GGCCCGC CUGAUGAG X CGAA AGGACUC	GAGUCCU A GCGGGCC

91	HCV-1418	1418	AGUAGGC CUGAUGAG X CGAA AGGCCCG	CGGGCCU U GCCUACU
92	HCV-1423	1423	GGAAUAG CUGAUGAG X CGAA AGGCAAG	CUUGCCU A CUAUUCC
93	HCV-1426	1426	CAUGGAA CUGAUGAG X CGAA AGUAGGC	GCCUACU A UUCCAUG
94	HCV-1428	1428	ACCAUGG CUGAUGAG X CGAA AUAGUAG	CUACUAU U CCAUGGU
95	HCV-1429	1429	CACCAUG CUGAUGAG X CGAA AAUAGUA	UACUAUU C CAUGGUG
96	HCV-1727	1727	ACUUGUC CUGAUGAG X CGAA AUGGAGC	GCUCCAU C GACAAGU
97	HCV-1735	1735	CUGAGCG CUGAUGAG X CGAA ACUUGUC	GACAAGU U CGCUCAG
98	HCV-1736	1736	CCUGAGC CUGAUGAG X CGAA AACUUGU	ACAAGUU C GCUCAGG
99	HCV-1740	1740	CAUCCCU CUGAUGAG X CGAA AGCGAAC	GUUCGCU C AGGGAUG
100	HCV-1757	1757	UAUAGGU CUGAUGAG X CGAA AUGGGGC	GCCCCAU C ACCUAUA
101	HCV-1762	1762	CUCGGUA CUGAUGAG X CGAA AGGUGAU	AUCACCU A UACCGAG
102	HCV-1795	1795	CCAGCAG CUGAUGAG X CGAA AAGGCCU	AGGCCUU A CUGCUGG
103	HCV-1806	1806	GGUGCGU CUGAUGAG X CGAA AUGCCAG	CUGGCAU U ACGCACC
104	HCV-1807	1807	AGGUGCG CUGAUGAG X CGAA AAUGCCA	UGGCAUU A CGCACCU
105	HCV-1815	1815	CACUGCC CUGAUGAG X CGAA AGGUGCG	CGCACCU C GGCAGUG
106	HCV-1827	1827	GGUACGA CUGAUGAG X CGAA ACCACAC	GUGUGGU A UCGUACC
107	HCV-1829	1829	CAGGUAC CUGAUGAG X CGAA AUACCAC	GUGGUAU C GUACCUG
108	HCV-1832	1832	ACGCAGG CUGAUGAG X CGAA ACGAUAC	GUAUCGU A CCUGCGU
109	HCV-1840	1840	CACCUGC CUGAUGAG X CGAA ACGCAGG	CCUGCGU C GCAGGUG
110	HCV-1854	1854	UACACUG CUGAUGAG X CGAA ACCACAC	GUGUGGU C CAGUGUA
111	HCV-1883	1883	CCACUAC CUGAUGAG X CGAA ACAGGGC	GCCCUGU U GUAGUGG
112	HCV-1886	1886	UCCCCAC CUGAUGAG X CGAA ACAACAG	CUGUUGU A GUGGGGA
113	HCV-1902	1902	CCGGACC CUGAUGAG X CGAA AUCGGUC	GACCGAU C GGUCCGG
114	HCV-1906	1906	GGCACCG CUGAUGAG X CGAA ACCGAUC	GAUCGGU C CGGUGCC
115	HCV-1917	1917	UUAUACG CUGAUGAG X CGAA AGGGGCA	UGCCCCU A CGUAUAA
116	HCV-1921	1921	CCAGUUA CUGAUGAG X CGAA ACGUAGG	CCUACGU A UAACUGG
117	HCV-1923	1923	CCCCAGU CUGAUGAG X CGAA AUACGUA	UACGUAU A ACUGGGG
118	HCV-1990	1990	ACAGCCA CUGAUGAG X CGAA ACCAGUU	AACUGGU U UGGCUGU
119	HCV-1991	1991	UACAGCC CUGAUGAG X CGAA AACCAGU	ACUGGUU U GGCUGUA
120	HCV-1998	1998	AUCCAUG CUGAUGAG X CGAA ACAGCCA	UGGCUGU A CAUGGAU
121	HCV-2043	2043	UUGCACG CUGAUGAG X CGAA AGGGCCC	GGGCCCU C CGUGCAA
122	HCV-2054	2054	CCCCCCC CUGAUGAG X CGAA AUGUUGC	GCAACAU C GGGGGGG
123	HCV-2063	2063	GGUUGCC CUGAUGAG X CGAA ACCCCCC	GGGGGGU C GGCAACC
124	HCV-2072	2072	UCAAGGU CUGAUGAG X CGAA AGGUUGC	GCAACCU C ACCUUGA
125	HCV-2077	2077	GCAGGUC CUGAUGAG X CGAA AGGUGAG	CUCACCU U GACCUGC
126	HCV-2121	2121	UUUGUGU CUGAUGAG X CGAA AGUGGCC	GGCCACU U ACACAAA
127	HCV-2122	2122	UUUUGUG CUGAUGAG X CGAA AAGUGGC	GCCACUU A CACAAAA
128	HCV-2137	2137	UGGCCCC CUGAUGAG X CGAA AGCCACA	UGUGGCU C GGGGCCA
129	HCV-2149	2149	AGGUGUU CUGAUGAG X CGAA ACCAUGG	CCAUGGU U AACACCU
130	HCV-2150	2150	UAGGUGU CUGAUGAG X CGAA AACCAUG	CAUGGUU A ACACCUA
131	HCV-2219	2219	CCUUAAA CUGAUGAG X CGAA AUGGUAA	UUACCAU C UUUAAGG
132	HCV-2221	2221	AACCUUA CUGAUGAG X CGAA AGAUGGU	ACCAUCU U UAAGGUU
133	HCV-2261	2261	CAGCACU CUGAUGAG X CGAA AGCCUGU	ACAGGCU U AGUGCUG
134	HCV-2262	2262	GCAGCAC CUGAUGAG X CGAA AAGCCUG	CAGGCUU A GUGCUGC
135	HCV-2295	2295	AGGUCGC CUGAUGAG X CGAA ACGCUCU	AGAGCGU U GCGACCU
136	HCV-2320	2320	GAGCUCC CUGAUGAG X CGAA AUCUGUC	GACAGAU C GGAGCUC
137	HCV-2327	2327	GCGGGCU CUGAUGAG X CGAA AGCUCCG	CGGAGCU C AGCCCGC

138	HCV-2344	2344	UGUCGUG CUGAUGAG X CGAA ACAGCAG	CUGCUGU C CACGACA
139	HCV-2417	2417	UCUGAUG CUGAUGAG X CGAA AGGUGGA	UCCACCU C CAUCAGA
140	HCV-2421	2421	AUGUUCU CUGAUGAG X CGAA AUGGAGG	CCUCCAU C AGAACAU
141	HCV-2429	2429	CGUCCAC CUGAUGAG X CGAA AUGUUCU	AGAACAU C GUGGACG
142	HCV-2534	2534	AGGCACA CUGAUGAG X CGAA ACGCGCG	CGCGCGU C UGUGCCU
143	HCV-2585	2585	GGUUCUC CUGAUGAG X CGAA AGGGCGG	CCGCCCU A GAGAACC
144	HCV-2600	2600	CGUUGAG CUGAUGAG X CGAA ACCACCA	UGGUGGU C CUCAACG
145	HCV-2603	2603	CCGCGUU CUGAUGAG X CGAA AGGACCA	UGGUCCU C AACGCGG
146	HCV-2671	2671	CUUGAUG CUGAUGAG X CGAA ACCAGGC	GCCUGGU A CAUCAAG
147	HCV-2675	2675	UGCCCUU CUGAUGAG X CGAA AUGUACC	GGUACAU C AAGGGCA
148	HCV-2690	2690	CCCCAGG CUGAUGAG X CGAA ACCAGCC	GGCUGGU C CCUGGGG
149	HCV-2704	2704	CAGAGCA CUGAUGAG X CGAA AUGCCGC	GCGGCAU A UGCUCUG
150	HCV-2709	2709	CCGUACA CUGAUGAG X CGAA AGCAUAU	AUAUGCU C UGUACGG
151	HCV-2713	2713	CACGCCG CUGAUGAG X CGAA ACAGAGC	GCUCUGU A CGGCGUG
152	HCV-2738	2738	CCAGCAG CUGAUGAG X CGAA AGCAGGA	UCCUGCU C CUGCUGG
153	HCV-2763	2763	AUGGCGU CUGAUGAG X CGAA AGCCCGU	ACGGGCU U ACGCCAU
154	HCV-2764	2764	CAUGGCG CUGAUGAG X CGAA AAGCCCG	CGGGCUU A CGCCAUG
155	HCV-2878	2878	GUAUUGU CUGAUGAG X CGAA ACCACCA	UGGUGGU U ACAAUAC
156	HCV-2879	2879	AGUAUUG CUGAUGAG X CGAA AACCACC	GGUGGUU A CAAUACU
157	HCV-2884	2884	GAUAAAG CUGAUGAG X CGAA AUUGUAA	UUACAAU A CUUUAUC
158	HCV-2887	2887	GGUGAUA CUGAUGAG X CGAA AGUAUUG	CAAUACU U UAUCACC
159	HCV-2888	2888	UGGUGAU CUGAUGAG X CGAA AAGUAUU	AAUACUU U AUCACCA
160	HCV-2910	2910	ACGCACA CUGAUGAG X CGAA AUGCGCC	GGCGCAU U UGUGCGU
161	HCV-2911	2911	CACGCAC CUGAUGAG X CGAA AAUGCGC	GCGCAUU U GUGCGUG
162	HCV-2924	2924	GAGGGGG CUGAUGAG X CGAA ACCCACA	UGUGGGU C CCCCCUC
163	HCV-2931	2931	ACAUUGA CUGAUGAG X CGAA AGGGGGG	CCCCCCU C UCAAUGU
164	HCV-2933	2933	GGACAUU CUGAUGAG X CGAA AGAGGGG	CCCCUCU C AAUGUCC
165	HCV-2939	2939	CCCCCCG CUGAUGAG X CGAA ACAUUGA	UCAAUGU C CGGGGGG
166	HCV-2958	2958	AGGAUGA CUGAUGAG X CGAA AGCAUCG	CGAUGCU A UCAUCCU
167	HCV-2960	2960	GGAGGAU CUGAUGAG X CGAA AUAGCAU	AUGCUAU C AUCCUCC
168	HCV-2963	2963	UGAGGAG CUGAUGAG X CGAA AUGAUAG	CUAUCAU C CUCCUCA
169	HCV-2966	2966	AUGUGAG CUGAUGAG X CGAA AGGAUGA	UCAUCCU C CUCACAU
170	HCV-2969	2969	CACAUGU CUGAUGAG X CGAA AGGAGGA	UCCUCCU C ACAUGUG
171	HCV-3059	3059	UCGCAGU CUGAUGAG X CGAA AUGGCAG	CUGCCAU A ACUGCGA
172	HCV-3138	3138	UGGACGU CUGAUGAG X CGAA AUGGCCU	AGGCCAU U ACGUCCA
173	HCV-3139	3139	UUGGACG CUGAUGAG X CGAA AAUGGCC	GGCCAUU A CGUCCAA
174	HCV-3143	3143	CCAUUUG CUGAUGAG X CGAA ACGUAAU	AUUACGU C CAAAUGG
175	HCV-3154	3154	CUUCAUG CUGAUGAG X CGAA AGGCCAU	AUGGCCU U CAUGAAG
176	HCV-3155	3155	GCUUCAU CUGAUGAG X CGAA AAGGCCA	UGGCCUU C AUGAAGC
177	HCV-3209	3209	AAUCCUG CUGAUGAG X CGAA AGCGGGG	CCCCGCU A CAGGAUU
178	HCV-3216	3216	UGGGCCC CUGAUGAG X CGAA AUCCUGU	ACAGGAU U GGGCCCA
179	HCV-3233	3233	GGUCUCG CUGAUGAG X CGAA AGGCCCG	CGGGCCU A CGAGACC
180	HCV-3242	3242	CCACCGC CUGAUGAG X CGAA AGGUCUC	GAGACCU U GCGGUGG
181	HCV-3263	3263	AGAAGAC CUGAUGAG X CGAA ACGGGCU	AGCCCGU C GUCUUCU
182	HCV-3266	3266	CAGAGAA CUGAUGAG X CGAA ACGACGG	CCGUCGU C UUCUCUG
183	HCV-3268	3268	GUCAGAG CUGAUGAG X CGAA AGACGAC	GUCGUCU U CUCUGAC
184	HCV-3290	3290	AGGUGAU CUGAUGAG X CGAA AUCUUGG	CCAAGAU C AUCACCU

185	HCV-3293	3293	CCCAGGU CUGAUGAG X CGAA AUGAUCU	AGAUCAU C ACCUGGG
186	HCV-3329	3329	CCAAGAU CUGAUGAG X CGAA AUGUCCC	GGGACAU C AUCUUGG
187	HCV-3332	3332	GUCCCAA CUGAUGAG X CGAA AUGAUGU	ACAUCAU C UUGGGAC
188	HCV-3334	3334	CAGUCCC CUGAUGAG X CGAA AGAUGAU	AUCAUCU U GGGACUG
189	HCV-3347	3347	GGGCGGA CUGAUGAG X CGAA ACGGGCA	UGCCCGU C UCCGCCC
190	HCV-3349	3349	UCGGGCG CUGAUGAG X CGAA AGACGGG	CCCGUCU C CGCCCGA
191	HCV-3371	3371	CCAGAAG CUGAUGAG X CGAA AUCUCCC	GGGAGAU A CUUCUGG
192	HCV-3416	3416	GGGCAAG CUGAUGAG X CGAA AGUCGCC	GGCGACU C CUUGCCC
193	HCV-3419	3419	UGGGGGC CUGAUGAG X CGAA AGGAGUC	GACUCCU U GCCCCCA
194	HCV-3428	3428	AGGCCGU CUGAUGAG X CGAA AUGGGGG	CCCCCAU C ACGGCCU
195	HCV-3482	3482	GGCCUGU CUGAUGAG X CGAA AGGCUAG	CUAGCCU C ACAGGCC
196	HCV-3518	3518	CCACUUG CUGAUGAG X CGAA ACCUCCC	GGGAGGU U CAAGUGG
197	HCV-3519	3519	ACCACUU CUGAUGAG X CGAA AACCUCC	GGAGGUU C AAGUGGU
198	HCV-3527	3527	CGGUGGA CUGAUGAG X CGAA ACCACUU	AAGUGGU U UCCACCG
199	HCV-3528	3528	GCGGUGG CUGAUGAG X CGAA AACCACU	AGUGGUU U CCACCGC
200	HCV-3529	3529	UGCGGUG CUGAUGAG X CGAA AAACCAC	GUGGUUU C CACCGCA
201	HCV-3576	3576	ACGGUCC CUGAUGAG X CGAA ACACACA	UGUGUGU U GGACCGU
202	HCV-3601	3601	GGUCUUU CUGAUGAG X CGAA AGCCGGC	GCCGGCU C AAAGACC
203	HCV-3611	3611	GGCCGGC CUGAUGAG X CGAA AGGGUCU	AGACCCU A GCCGGCC
204	HCV-3684	3684	GCCCCGG CUGAUGAG X CGAA AGGCGCA	UGCGCCU C CCGGGGC
205	HCV-3696	3696	GUAAGGG CUGAUGAG X CGAA ACGCGCC	GGCGCGU U CCCUUAC
206	HCV-3697	3697	UGUAAGG CUGAUGAG X CGAA AACGCGC	GCGCGUU C CCUUACA
207	HCV-3701	3701	AUGGUGU CUGAUGAG X CGAA AGGGAAC	GUUCCCU U ACACCAU
208	HCV-3702	3702	CAUGGUG CUGAUGAG X CGAA AAGGGAA	UUCCCUU A CACCAUG
209	HCV-3724	3724	GAGGUCC CUGAUGAG X CGAA AGCUACC	GGUAGCU C GGACCUC
210	HCV-3731	3731	CCAGAUA CUGAUGAG X CGAA AGGUCCG	CGGACCU C UAUCUGG
211	HCV-3733	3733	GACCAGA CUGAUGAG X CGAA AGAGGUC	GACCUCU A UCUGGUC
212	HCV-3735	3735	GUGACCA CUGAUGAG X CGAA AUAGAGG	CCUCUAU C UGGUCAC
213	HCV-3740	3740	GUCUCGU CUGAUGAG X CGAA ACCAGAU	AUCUGGU C ACGAGAC
214	HCV-3761	3761	GCACCGG CUGAUGAG X CGAA AUGACGU	ACGUCAU U CCGGUGC
215	HCV-3762	3762	CGCACCG CUGAUGAG X CGAA AAUGACG	CGUCAUU C CGGUGCG
216	HCV-3786	3786	CUCCCCC CUGAUGAG X CGAA ACCGUCA	UGACGGU C GGGGGAG
217	HCV-3797	3797	GGGACAG CUGAUGAG X CGAA AGGCUCC	GGAGCCU A CUGUCCC
218	HCV-3802	3802	UCUGGGG CUGAUGAG X CGAA ACAGUAG	CUACUGU C CCCCAGA
219	HCV-3835	3835	GCCACCC CUGAUGAG X CGAA AAGAGCC	GGCUCUU C GGGUGGC
220	HCV-3851	3851	AAGGGCA CUGAUGAG X CGAA AGCAGUG	CACUGCU C UGCCCUU
221	HCV-3858	3858	UGCCCCG CUGAUGAG X CGAA AGGGCAG	CUGCCCU U CGGGGCA
222	HCV-3859	3859	GUGCCCC CUGAUGAG X CGAA AAGGGCA	UGCCCUU C GGGGCAC
223	HCV-3872	3872	AGAUGCC CUGAUGAG X CGAA ACAGCGU	ACGCUGU A GGCAUCU
224	HCV-3878	3878	CCCGGAA CUGAUGAG X CGAA AUGCCUA	UAGGCAU C UUCCGGG
225	HCV-3880	3880	AGCCCGG CUGAUGAG X CGAA AGAUGCC	GGCAUCU U CCGGGCU
226	HCV-3881	3881	CAGCCCG CUGAUGAG X CGAA AAGAUGC	GCAUCUU C CGGGCUG
227	HCV-3908	3908	CCUUCGC CUGAUGAG X CGAA ACCCCCC	GGGGGGU U GCGAAGG
228	HCV-4056	4056	GGCACUU CUGAUGAG X CGAA AGUGCUC	GAGCACU A AAGUGCC
229	HCV-4072	4072	GGCUGCG CUGAUGAG X CGAA ACGCAGC	GCUGCGU A CGCAGCC
230	HCV-4087	4087	UACCUUG CUGAUGAG X CGAA ACCCUUG	CAAGGGU A CAAGGUA
231	HCV-4115	4115	UGGCGGC CUGAUGAG X CGAA ACAGAUG	CAUCUGU U GCCGCCA

232	HCV-4175	4175	CAGUUCU CUGAUGAG X CGAA AUGUUGG	CCAACAU C AGAACUG
233	HCV-4187	4187	UGGUCCU CUGAUGAG X CGAA ACCCCAG	CUGGGGU A AGGACCA
234	HCV-4228	4228	CUUACCA CUGAUGAG X CGAA AGGUGGA	UCCACCU A UGGUAAG
235	HCV-4233	4233	AGGAACU CUGAUGAG X CGAA ACCAUAG	CUAUGGU A AGUUCCU
236	HCV-4237	4237	GGCAAGG CUGAUGAG X CGAA ACUUACC	GGUAAGU U CCUUGCC
237	HCV-4238	4238	CGGCAAG CUGAUGAG X CGAA AACUUAC	GUAAGUU C CUUGCCG
238	HCV-4241	4241	CGUCGGC CUGAUGAG X CGAA AGGAACU	AGUUCCU U GCCGACG
239	HCV-4280	4280	CACAUAU CUGAUGAG X CGAA AUGAUAU	AUAUCAU A AUAUGUG
240	HCV-4283	4283	CAUCACA CUGAUGAG X CGAA AUUAUGA	UCAUAAU A UGUGAUG
241	HCV-4337	4337	GGUCCAG CUGAUGAG X CGAA ACUGUGC	GCACAGU C CUGGACC
242	HCV-4370	4370	GCACGAC CUGAUGAG X CGAA AGCCGCG	CGCGGCU C GUCGUGC
243	HCV-4373	4373	CGAGCAC CUGAUGAG X CGAA ACGAGCC	GGCUCGU C GUGCUCG
244	HCV-4379	4379	CGGUGGC CUGAUGAG X CGAA AGCACGA	UCGUGCU C GCCACCG
245	HCV-4425	4425	UCCUCAA CUGAUGAG X CGAA AUUUGGG	CCCAAAU A UUGAGGA
246	HCV-4444	4444	AGUGUUG CUGAUGAG X CGAA ACAGAGC	GCUCUGU C CAACACU
247	HCV-4460	4460	AGAAGGG CUGAUGAG X CGAA AUCUCUC	GAGAGAU C CCCUUCU
248	HCV-4481	4481	CGAGGGG CUGAUGAG X CGAA AUGGCCU	AGGCCAU C CCCCUCG
249	HCV-4487	4487	UGGCCUC CUGAUGAG X CGAA AGGGGGA	UCCCCCU C GAGGCCA
250	HCV-4496	4496	CCCCCUU CUGAUGAG X CGAA AUGGCCU	AGGCCAU C AAGGGGG
251	HCV-4528	4528	CUUCUUG CUGAUGAG X CGAA AGUGGCA	UGCCACU C CAAGAAG
252	HCV-4577	4577	CGGCAUU CUGAUGAG X CGAA AUUCCGA	UCGGAAU C AAUGCCG
253	HCV-4586	4586	AAUACGC CUGAUGAG X CGAA ACGGCAU	AUGCCGU A GCGUAUU
254	HCV-4591	4591	CCGGUAA CUGAUGAG X CGAA ACGCUAC	GUAGCGU A UUACCGG
255	HCV-4593	4593	CCCCGGU CUGAUGAG X CGAA AUACGCU	AGCGUAU U ACCGGGG
256	HCV-4594	4594	ACCCCGG CUGAUGAG X CGAA AAUACGC	GCGUAUU A CCGGGGU
257	HCV-4616	4616	UCGGUAU CUGAUGAG X CGAA ACGGACA	UGUCCGU C AUACCGA
258	HCV-4619	4619	UAGUCGG CUGAUGAG X CGAA AUGACGG	CCGUCAU A CCGACUA
259	HCV-4626	4626	UCUCCGC CUGAUGAG X CGAA AGUCGGU	ACCGACU A GCGGAGA
260	HCV-4672	4672	ACCGGUG CUGAUGAG X CGAA AGCCCGU	ACGGGCU A CACCGGU
261	HCV-4697	4697	UGCAGUC CUGAUGAG X CGAA AUCACCG	CGGUGAU C GACUGCA
262	HCV-4789	4789	UGAGCGC CUGAUGAG X CGAA ACACCGC	GCGGUGU C GCGCUCA
263	HCV-4795	4795	CCGUUGU CUGAUGAG X CGAA AGCGCGA	UCGCGCU C ACAACGG
264	HCV-4920	4920	UCAUACC CUGAUGAG X CGAA AGCACAG	CUGUGCU U GGUAUGA
265	HCV-4924	4924	GAGCUCA CUGAUGAG X CGAA ACCAAGC	GCUUGGU A UGAGCUC
266	HCV-4931	4931	CGGGCGU CUGAUGAG X CGAA AGCUCAU	AUGAGCU C ACGCCCG
267	HCV-4947	4947	CUGACUG CUGAUGAG X CGAA AGUCUCA	UGAGACU A CAGUCAG
268	HCV-4952	4952	GCAACCU CUGAUGAG X CGAA ACUGUAG	CUACAGU C AGGUUGC
269	HCV-4957	4957	AGCCCGC CUGAUGAG X CGAA ACCUGAC	GUCAGGU U GCGGGCU
270	HCV-4965	4965	UUCAGGU CUGAUGAG X CGAA AGCCCGC	GCGGGCU U ACCUGAA
271	HCV-4966	4966	AUUCAGG CUGAUGAG X CGAA AAGCCCG	CGGGCUU A CCUGAAU
272	HCV-4974	4974	CCUGGUG CUGAUGAG X CGAA AUUCAGG	CCUGAAU A CACCAGG
273	HCV-4984	4984	GACGGGC CUGAUGAG X CGAA ACCCUGG	CCAGGGU U GCCCGUC
274	HCV-4991	4991	CCUGGCA CUGAUGAG X CGAA ACGGGCA	UGCCCGU C UGCCAGG
275	HCV-5004	5004	AACUCCA CUGAUGAG X CGAA AUGGUCC	GGACCAU C UGGAGUU
276	HCV-5102	5102	GGUAUGC CUGAUGAG X CGAA ACCAGGU	ACCUGGU A GCAUACC
277	HCV-5107	5107	GGCUUGG CUGAUGAG X CGAA AUGCUAC	GUAGCAU A CCAAGCC
278	HCV-5133	5133	GGAGCCU CUGAUGAG X CGAA AGCCCUG	CAGGGCU C AGGCUCC

279	HCV-5218	5218	UAGCCUA CUGAUGAG X CGAA ACAGCAG	CUGCUGU A UAGGCUA
280	HCV-5220	5220	CCUAGCC CUGAUGAG X CGAA AUACAGC	GCUGUAU A GGCUAGG
281	HCV-5306	5306	UAGUGAC CUGAUGAG X CGAA ACCUCCA	UGGAGGU C GUCACUA
282	HCV-5309	5309	UGCUAGU CUGAUGAG X CGAA ACGACCU	AGGUCGU C ACUAGCA
283	HCV-5313	5313	CAGGUGC CUGAUGAG X CGAA AGUGACG	CGUCACU A GCACCUG
284	HCV-5330	5330	CUCCGCC CUGAUGAG X CGAA ACCAGCA	UGCUGGU A GGCGGAG
285	HCV-5339	5339	CUGCAAG CUGAUGAG X CGAA ACUCCGC	GCGGAGU C CUUGCAG
286	HCV-5342	5342	GAGCUGC CUGAUGAG X CGAA AGGACUC	GAGUCCU U GCAGCUC
287	HCV-5359	5359	CAGGCAA CUGAUGAG X CGAA AUGCGGC	GCCGCAU A UUGCCUG
288	HCV-5361	5361	GUCAGGC CUGAUGAG X CGAA AUAUGCG	CGCAUAU U GCCUGAC
289	HCV-5376	5376	ACCACAC CUGAUGAG X CGAA ACCGGUU	AACCGGU A GUGUGGU
290	HCV-5399	5399	ACAAAAU CUGAUGAG X CGAA AUCCUAC	GUAGGAU C AUUUUGU
291	HCV-5423	5423	CGGGAAC CUGAUGAG X CGAA ACAGCCG	CGGCUGU U GUUCCCG
292	HCV-5426	5426	UGUCGGG CUGAUGAG X CGAA ACAACAG	CUGUUGU U CCCGACA
293	HCV-5427	5427	CUGUCGG CUGAUGAG X CGAA AACAACA	UGUUGUU C CCGACAG
294	HCV-5524	5524	CUGCUUG CUGAUGAG X CGAA ACUGCUC	GAGCAGU U CAAGCAG
295	HCV-5525	5525	UCUGCUU CUGAUGAG X CGAA AACUGCU	AGCAGUU C AAGCAGA
296	HCV-5583	5583	ACCACGG CUGAUGAG X CGAA AGCAGCG	CGCUGCU C CCGUGGU
297	HCV-5596	5596	CCACCUG CUGAUGAG X CGAA ACUCCAC	GUGGAGU C CAGGUGG
298	HCV-5612	5612	AGGCCUC CUGAUGAG X CGAA AGGGCCC	GGGCCCU U GAGGCCU
299	HCV-5620	5620	UGCCCAG CUGAUGAG X CGAA AGGCCUC	GAGGCCU U CUGGGCA
300	HCV-5621	5621	UUGCCCA CUGAUGAG X CGAA AAGGCCU	AGGCCUU C UGGGCAA
301	HCV-5674	5674	AGUGGAU CUGAUGAG X CGAA AGCCUGC	GCAGGCU U AUCCACU
302	HCV-5675	5675	GAGUGGA CUGAUGAG X CGAA AAGCCUG	CAGGCUU A UCCACUC
303	HCV-5767	5767	GAUGUUG CUGAUGAG X CGAA ACAGGAG	CUCCUGU U CAACAUC
304	HCV-5768	5768	AGAUGUU CUGAUGAG X CGAA AACAGGA	UCCUGUU C AACAUCU
305	HCV-5801	5801	GAGGAGC CUGAUGAG X CGAA AGUUGAG	CUCAACU C GCUCCUC
306	HCV-5805	5805	CUGGGAG CUGAUGAG X CGAA AGCGAGU	ACUCGCU C CUCCCAG
307	HCV-5821	5821	GAAGGCC CUGAUGAG X CGAA AAGCAGC	GCUGCUU C GGCCUUC
308	HCV-5827	5827	GCCCACG CUGAUGAG X CGAA AGGCCGA	UCGGCCU U CGUGGGC
309	HCV-5828	5828	CGCCCAC CUGAUGAG X CGAA AAGGCCG	CGGCCUU C GUGGGCG
310	HCV-5843	5843	CACCGGC CUGAUGAG X CGAA AUGCCGG	CCGGCAU U GCCGGUG
311	HCV-5858	5858	UGCUGCC CUGAUGAG X CGAA AUGGCCG	CGGCCAU U GGCAGCA
312	HCV-5867	5867	CAAGGCC CUGAUGAG X CGAA AUGCUGC	GCAGCAU A GGCCUUG
313	HCV-5873	5873	CCUUCCC CUGAUGAG X CGAA AGGCCUA	UAGGCCU U GGGAAGG
314	HCV-5905	5905	CGCUCCA CUGAUGAG X CGAA AGCCCGC	GCGGGCU A UGGAGCG
315	HCV-5930	5930	AAGCCAC CUGAUGAG X CGAA AGUGCAC	GUGCACU C GUGGCUU
316	HCV-5937	5937	ACCUUAA CUGAUGAG X CGAA AGCCACG	CGUGGCU U UUAAGGU
317	HCV-5938	5938	GACCUUA CUGAUGAG X CGAA AAGCCAC	GUGGCUU U UAAGGUC
318	HCV-5939	5939	UGACCUU CUGAUGAG X CGAA AAAGCCA	UGGCUUU U AAGGUCA
319	HCV-5940	5940	AUGACCU CUGAUGAG X CGAA AAAAGCC	GGCUUUU A AGGUCAU
320	HCV-5945	5945	CGCUCAU CUGAUGAG X CGAA ACCUUAA	UUAAGGU C AUGAGCG
321	HCV-5965	5965	CUCGGCG CUGAUGAG X CGAA AGGGCGC	GCGCCCU C CGCCGAG
322	HCV-5981	5981	GCAAGUU CUGAUGAG X CGAA ACCAGGU	ACCUGGU U AACUUGC
323	HCV-5982	5982	AGCAAGU CUGAUGAG X CGAA AACCAGG	CCUGGUU A ACUUGCU
324	HCV-5990	5990	UGGCAGG CUGAUGAG X CGAA AGCAAGU	ACUUGCU C CCUGCCA
325	HCV-6004	6004	GCCGGGG CUGAUGAG X CGAA AGAGGAU	AUCCUCU C CCCCGGC

326	HCV-6020	6020	CCCCGAC CUGAUGAG X CGAA ACCAGGG	CCCUGGU C GUCGGGG
327	HCV-6023	6023	CGACCCC CUGAUGAG X CGAA ACGACCA	UGGUCGU C GGGGUCG
328	HCV-6029	6029	CACACAC CUGAUGAG X CGAA ACCCCGA	UCGGGGU C GUGUGUG
329	HCV-6044	6044	GACGCAG CUGAUGAG X CGAA AUUGCUG	CAGCAAU C CUGCGUC
330	HCV-6051	6051	ACGUGCC CUGAUGAG X CGAA ACGCAGG	CCUGCGU C GGCACGU
331	HCV-6106	6106	CGAAGCG CUGAUGAG X CGAA ACGCUAU	AUAGCGU U CGCUUCG
332	HCV-6107	6107	GCGAAGC CUGAUGAG X CGAA AACGCUA	UAGCGUU C GCUUCGC
333	HCV-6111	6111	CCCCGCG CUGAUGAG X CGAA AGCGAAC	GUUCGCU U CGCGGGG
334	HCV-6413	6413	UUUGCAU CUGAUGAG X CGAA AUGCCGU	ACGGCAU C AUGCAAA
335	HCV-6574	6574	CCUGGAA CUGAUGAG X CGAA AGUUCGG	CCGAACU A UUCCAGG
336	HCV-6576	6576	GCCCUGG CUGAUGAG X CGAA AUAGUUC	GAACUAU U CCAGGGC
337	HCV-6577	6577	CGCCCUG CUGAUGAG X CGAA AAUAGUU	AACUAUU C CAGGGCG
338	HCV-6637	6637	GUAGUGG CUGAUGAG X CGAA AGUCCCC	GGGGACU U CCACUAC
339	HCV-6638	6638	CGUAGUG CUGAUGAG X CGAA AAGUCCC	GGGACUU C CACUACG
340	HCV-6643	6643	CGUCACG CUGAUGAG X CGAA AGUGGAA	UUCCACU A CGUGACG
341	HCV-6671	6671	GGCAUUU CUGAUGAG X CGAA ACGUUGU	ACAACGU A AAAUGCC
342	HCV-6703	6703	GGUGAAG CUGAUGAG X CGAA AUUCGGG	CCCGAAU U CUUCACC
343	HCV-6704	6704	CGGUGAA CUGAUGAG X CGAA AAUUCGG	CCGAAUU C UUCACCG
344	HCV-6706	6706	UUCGGUG CUGAUGAG X CGAA AGAAUUC	GAAUUCU U CACCGAA
345	HCV-6707	6707	AUUCGGU CUGAUGAG X CGAA AAGAAUU	AAUUCUU C ACCGAAU
346	HCV-6715	6715	CCCGUCC CUGAUGAG X CGAA AUUCGGU	ACCGAAU U GGACGGG
347	HCV-6730	6730	CCUGUGC CUGAUGAG X CGAA ACCGCAC	GUGCGGU U GCACAGG
348	HCV-6739	6739	CGGAGCG CUGAUGAG X CGAA ACCUGUG	CACAGGU A CGCUCCG
349	HCV-6744	6744	CACGCCG CUGAUGAG X CGAA AGCGUAC	GUACGCU C CGGCGUG
350	HCV-6759	6759	CGUAGGA CUGAUGAG X CGAA AGGUCUG	CAGACCU C UCCUACG
351	HCV-6761	6761	CCCGUAG CUGAUGAG X CGAA AGAGGUC	GACCUCU C CUACGGG
352	HCV-6764	6764	CCUCCCG CUGAUGAG X CGAA AGGAGAG	CUCUCCU A CGGGAGG
353	HCV-6776	6776	GGAAUGU CUGAUGAG X CGAA ACAUCCU	AGGAUGU C ACAUUCC
354	HCV-6782	6782	CGACCUG CUGAUGAG X CGAA AAUGUGA	UCACAUU C CAGGUCG
355	HCV-6788	6788	UGAGCCC CUGAUGAG X CGAA ACCUGGA	UCCAGGU C GGGCUCA
356	HCV-6794	6794	AUUGGUU CUGAUGAG X CGAA AGCCCGA	UCGGGCU C AACCAAU
357	HCV-6802	6802	AACCAGG CUGAUGAG X CGAA AUUGGUU	AACCAAU A CCUGGUU
358	HCV-6809	6809	GUGACCC CUGAUGAG X CGAA ACCAGGU	ACCUGGU U GGGUCAC
359	HCV-6814	6814	GAGCUGU CUGAUGAG X CGAA ACCCAAC	GUUGGGU C ACAGCUC
360	HCV-6821	6821	CGCAUGG CUGAUGAG X CGAA AGCUGUG	CACAGCU C CCAUGCG
361	HCV-6906	6906	GCCAGCC CUGAUGAG X CGAA ACGUUUA	UAAACGU A GGCUGGC
362	HCV-6922	6922	GGGGGGA CUGAUGAG X CGAA ACCCCCU	AGGGGGU C UCCCCCC
363	HCV-6924	6924	GAGGGGG CUGAUGAG X CGAA AGACCCC	GGGGUCU C CCCCCUC
364	HCV-6931	6931	GGCCAAG CUGAUGAG X CGAA AGGGGGG	CCCCCCU C CUUGGCC
365	HCV-6934	6934	GCUGGCC CUGAUGAG X CGAA AGGAGGG	CCCUCCU U GGCCAGC
366	HCV-6943	6943	AGCUGAA CUGAUGAG X CGAA AGCUGGC	GCCAGCU C UUCAGCU
367	HCV-6958	6958	CGCAGAC CUGAUGAG X CGAA AUUGGCU	AGCCAAU U GUCUGCG
368	HCV-6961	6961	AGGCGCA CUGAUGAG X CGAA ACAAUUG	CAAUUGU C UGCGCCU
369	HCV-7034	7034	GCCACAG CUGAUGAG X CGAA AGGUUGG	CCAACCU C CUGUGGC
370	HCV-7118	7118	CCGCUCG CUGAUGAG X CGAA AGCGGGU	ACCCGCU U CGAGCGG
371	HCV-7119	7119	UCCGCUC CUGAUGAG X CGAA AAGCGGG	CCCGCUU C GAGCGGA
372	HCV-7145	7145	CAACGGA CUGAUGAG X CGAA ACUUCCC	GGGAAGU A UCCGUUG

373	HCV-7195	7195	UAUGGGC CUGAUGAG X CGAA ACGCGGG	CCCGCGU U GCCCAUA
374	HCV-7202	7202	GUGCCCA CUGAUGAG X CGAA AUGGGCA	UGCCCAU A UGGGCAC
375	HCV-7218	7218	GGGUUGU CUGAUGAG X CGAA AUCCGGG	CCCGGAU U ACAACCC
376	HCV-7219	7219	AGGGUUG CUGAUGAG X CGAA AAUCCGG	CCGGAUU A CAACCCU
377	HCV-7234	7234	GGACUCU CUGAUGAG X CGAA ACAGUGG	CCACUGU U AGAGUCC
378	HCV-7235	7235	AGGACUC CUGAUGAG X CGAA AACAGUG	CACUGUU A GAGUCCU
379	HCV-7251	7251	UAGUCCG CUGAUGAG X CGAA ACUUUUC	GAAAAGU C CGGACUA
380	HCV-7258	7258	AGGGACG CUGAUGAG X CGAA AGUCCGG	CCGGACU A CGUCCCU
381	HCV-7262	7262	CCGGAGG CUGAUGAG X CGAA ACGUAGU	ACUACGU C CCUCCGG
382	HCV-7266	7266	ACCGCCG CUGAUGAG X CGAA AGGGACG	CGUCCCU C CGGCGGU
383	HCV-7288	7288	AGGCGGC CUGAUGAG X CGAA AUGGGCA	UGCCCAU U GCCGCCU
384	HCV-7296	7296	CCCGUGG CUGAUGAG X CGAA AGGCGGC	GCCGCCU A CCACGGG
385	HCV-7354	7354	CACGGUG CUGAUGAG X CGAA ACUCUGU	ACAGAGU C CACCGUG
386	HCV-7386	7386	GUCUUAG CUGAUGAG X CGAA AGCCAGC	GCUGGCU A CUAAGAC
387	HCV-7389	7389	AAAGUCU CUGAUGAG X CGAA AGUAGCC	GGCUACU A AGACUUU
388	HCV-7395	7395	CUGCCGA CUGAUGAG X CGAA AGUCUUA	UAAGACU U UCGGCAG
389	HCV-7396	7396	GCUGCCG CUGAUGAG X CGAA AAGUCUU	AAGACUU U CGGCAGC
390	HCV-7397	7397	AGCUGCC CUGAUGAG X CGAA AAAGUCU	AGACUUU C GGCAGCU
391	HCV-7411	7411	GGCCGAC CUGAUGAG X CGAA AUCCGGA	UCCGGAU C GUCGGCC
392	HCV-7414	7414	AACGGCC CUGAUGAG X CGAA ACGAUCC	GGAUCGU C GGCCGUU
393	HCV-7421	7421	CGCUGUC CUGAUGAG X CGAA ACGGCCG	CGGCCGU U GACAGCG
394	HCV-7498	7498	CAUGGAG CUGAUGAG X CGAA AGUACGA	UCGUACU C CUCCAUG
395	HCV-7501	7501	GGGCAUG CUGAUGAG X CGAA AGGAGUA	UACUCCU C CAUGCCC
396	HCV-7514	7514	CCCCCUC CUGAUGAG X CGAA AGGGGGG	CCCCCCU U GAGGGGG
397	HCV-7539	7539	UCGCUGA CUGAUGAG X CGAA AUCAGGG	CCCUGAU C UCAGCGA
398	HCV-7541	7541	CGUCGCU CUGAUGAG X CGAA AGAUCAG	CUGAUCU C AGCGACG
399	HCV-7552	7552	AGACCAA CUGAUGAG X CGAA ACCCGUC	GACGGGU C UUGGUCU
400	HCV-7554	7554	GUAGACC CUGAUGAG X CGAA AGACCCG	CGGGUCU U GGUCUAC
401	HCV-7558	7558	CACGGUA CUGAUGAG X CGAA ACCAAGA	UCUUGGU C UACCGUG
402	HCV-7560	7560	CUCACGG CUGAUGAG X CGAA AGACCAA	UUGGUCU A CCGUGAG
403	HCV-7589	7589	AGCAGAC CUGAUGAG X CGAA AUGUCGU	ACGACAU C GUCUGCU
404	HCV-7592	7592	AGCAGCA CUGAUGAG X CGAA ACGAUGU	ACAUCGU C UGCUGCU
405	HCV-7600	7600	GGACAUU CUGAUGAG X CGAA AGCAGCA	UGCUGCU C AAUGUCC
406	HCV-7606	7606	UGUGUAG CUGAUGAG X CGAA ACAUUGA	UCAAUGU C CUACACA
407	HCV-7667	7667	ACGCGUU CUGAUGAG X CGAA AUGGGCA	UGCCCAU C AACGCGU
408	HCV-7723	7723	ACUGCGG CUGAUGAG X CGAA AUGUUGU	ACAACAU C CCGCAGU
409	HCV-7775	7775	CGUCCAG CUGAUGAG X CGAA ACUUGCA	UGCAAGU C CUGGACG
410	HCV-7789	7789	GUCCCGG CUGAUGAG X CGAA AGUGGUC	GACCACU A CCGGGAC
411	HCV-7839	7839	AGAAGUU CUGAUGAG X CGAA AGCCUUA	UAAGGCU A AACUUCU
412	HCV-7847	7847	CUACGGA CUGAUGAG X CGAA AGAAGUU	AACUUCU A UCCGUAG
413	HCV-7849	7849	UUCUACG CUGAUGAG X CGAA AUAGAAG	CUUCUAU C CGUAGAA
414	HCV-7853	7853	CUUCUUC CUGAUGAG X CGAA ACGGAUA	UAUCCGU A GAAGAAG
415	HCV-7894	7894	AAAUUUA CUGAUGAG X CGAA AUUUGGC	GCCAAAU C UAAAUUU
416	HCV-7896	7896	CCAAAUU CUGAUGAG X CGAA AGAUUUG	CAAAUCU A AAUUUGG
417	HCV-7900	7900	AUAGCCA CUGAUGAG X CGAA AUUUAGA	UCUAAAU U UGGCUAU
418	HCV-7901	7901	CAUAGCC CUGAUGAG X CGAA AAUUUAG	CUAAAUU U GGCUAUG
419	HCV-7906	7906	UGCCCCA CUGAUGAG X CGAA AGCCAAA	UUUGGCU A UGGGGCA

420	HCV-7955	7955	CGGAGCG CUGAUGAG X CGAA AUGUGGU	ACCACAU C CGCUCCG
421	HCV-7960	7960	CCACACG CUGAUGAG X CGAA AGCGGAU	AUCCGCU C CGUGUGG
422	HCV-8075	8075	AUACGAU CUGAUGAG X CGAA AGGCGAG	CUCGCCU U AUCGUAU
423	HCV-8076	8076	AAUACGA CUGAUGAG X CGAA AAGGCGA	UCGCCUU A UCGUAUU
424	HCV-8078	8078	GGAAUAC CUGAUGAG X CGAA AUAAGGC	GCCUUAU C GUAUUCC
425	HCV-8170	8170	GAAUCCG CUGAUGAG X CGAA ACGAGGA	UCCUCGU A CGGAUUC
426	HCV-8176	8176	GUACUGG CUGAUGAG X CGAA AUCCGUA	UACGGAU U CCAGUAC
427	HCV-8182	8182	AGGAGAG CUGAUGAG X CGAA ACUGGAA	UUCCAGU A CUCUCCU
428	HCV-8187	8187	UGCCCAG CUGAUGAG X CGAA AGAGUAC	GUACUCU C CUGGGCA
429	HCV-8201	8201	GGAACUC CUGAUGAG X CGAA ACCCGCU	AGCGGGU U GAGUUCC
430	HCV-8206	8206	CACCAGG CUGAUGAG X CGAA ACUCAAC	GUUGAGU U CCUGGUG
431	HCV-8207	8207	UCACCAG CUGAUGAG X CGAA AACUCAA	UUGAGUU C CUGGUGA
432	HCV-8227	8227	UUUCUUU CUGAUGAG X CGAA AUUUCCA	UGGAAAU C AAAGAAA
433	HCV-8357	8357	GCGACUU CUGAUGAG X CGAA AUGGCCU	AGGCCAU A AAGUCGC
434	HCV-8362	8362	CGUGAGC CUGAUGAG X CGAA ACUUUAU	AUAAAGU C GCUCACG
435	HCV-8366	8366	GCUCCGU CUGAUGAG X CGAA AGCGACU	AGUCGCU C ACGGAGC
436	HCV-8378	8378	CGAUGUA CUGAUGAG X CGAA AGCCGCU	AGCGGCU C UACAUCG
437	HCV-8380	8380	CCCGAUG CUGAUGAG X CGAA AGAGCCG	CGGCUCU A CAUCGGG
438	HCV-8384	8384	GGCCCCC CUGAUGAG X CGAA AUGUAGA	UCUACAU C GGGGGCC
439	HCV-8424	8424	CGGCGAU CUGAUGAG X CGAA ACCGCAG	CUGCGGU U AUCGCCG
440	HCV-8425	8425	CCGGCGA CUGAUGAG X CGAA AACCGCA	UGCGGUU A UCGCCGG
441	HCV-8427	8427	CACCGGC CUGAUGAG X CGAA AUAACCG	CGGUUAU C GCCGGUG
442	HCV-8460	8460	CCGCAGC CUGAUGAG X CGAA AGUCGUC	GACGACU A GCUGCGG
443	HCV-8508	8508	GCAGCUC CUGAUGAG X CGAA ACAGGCC	GGCCUGU C GAGCUGC
444	HCV-8522	8522	AGUCCUG CUGAUGAG X CGAA AGCUUUG	CAAAGCU C CAGGACU
445	HCV-8540	8540	CGUUCAC CUGAUGAG X CGAA AGCAUCG	CGAUGCU C GUGAACG
446	HCV-8558	8558	UAACGAC CUGAUGAG X CGAA AGGUCGU	ACGACCU U GUCGUUA
447	HCV-8561	8561	AGAUAAC CUGAUGAG X CGAA ACAAGGU	ACCUUGU C GUUAUCU
448	HCV-8564	8564	CACAGAU CUGAUGAG X CGAA ACGACAA	UUGUCGU U AUCUGUG
449	HCV-8638	8638	GGGGGCA CUGAUGAG X CGAA AGUACCU	AGGUACU C UGCCCCC
450	HCV-8671	8671	CAAGUCG CUGAUGAG X CGAA AUUCUGG	CCAGAAU A CGACUUG
451	HCV-8698	8698	GUUGGAG CUGAUGAG X CGAA AGCAUGA	UCAUGCU C CUCCAAC
452	HCV-8701	8701	CACGUUG CUGAUGAG X CGAA AGGAGCA	UGCUCCU C CAACGUG
453	HCV-8728	8728	UUUGCCG CUGAUGAG X CGAA AUGCGUC	GACGCAU C CGGCAAA
454	HCV-8774	8774	CCCGUGC CUGAUGAG X CGAA AGGGGGG	CCCCCCU U GCACGGG
455	HCV-8842	8842	GGGCGCA CUGAUGAG X CGAA ACAUGAU	AUCAUGU A UGCGCCC
456	HCV-8854	8854	UGCCCAU CUGAUGAG X CGAA AGGUGGG	CCCACCU U AUGGGCA
457	HCV-8855	8855	UUGCCCA CUGAUGAG X CGAA AAGGUGG	CCACCUU A UGGGCAA
458	HCV-8871	8871	GUCAUCA CUGAUGAG X CGAA AAUCAUC	GAUGAUU U UGAUGAC
459	HCV-8880	8880	AAGAAGU CUGAUGAG X CGAA AGUCAUC	GAUGACU C ACUUCUU
460	HCV-8931	8931	AUCUGAC CUGAUGAG X CGAA AUCCAGG	CCUGGAU U GUCAGAU
461	HCV-8934	8934	UAGAUCU CUGAUGAG X CGAA ACAAUCC	GGAUUGU C AGAUCUA
462	HCV-8939	8939	CCCCGUA CUGAUGAG X CGAA AUCUGAC	GUCAGAU C UACGGGG
463	HCV-8941	8941	GGCCCCG CUGAUGAG X CGAA AGAUCUG	CAGAUCU A CGGGGCC
464	HCV-9065	9065	GUUUCCU CUGAUGAG X CGAA AGGCAUG	CAUGCCU C AGGAAAC
465	HCV-9074	9074	GUACCCC CUGAUGAG X CGAA AGUUUCC	GGAAACU U GGGGUAC
466	HCV-9080	9080	AGGGCGG CUGAUGAG X CGAA ACCCCAA	UUGGGGU A CCGCCCU

467	HCV-9088	9088	GACUCGC CUGAUGAG X CGAA AGGGCGG	CCGCCCU U GCGAGUC
468	HCV-9095	9095	GUCUCCA CUGAUGAG X CGAA ACUCGCA	UGCGAGU C UGGAGAC
469	HCV-9119	9119	UAGCGCG CUGAUGAG X CGAA ACACUUC	GAAGUGU C CGCGCUA
470	HCV-9126	9126	AGUAGCC CUGAUGAG X CGAA AGCGCGG	CCGCGCU A GGCUACU
471	HCV-9131	9131	GGGACAG CUGAUGAG X CGAA AGCCUAG	CUAGGCU A CUGUCCC
472	HCV-9136	9136	CCCUUGG CUGAUGAG X CGAA ACAGUAG	CUACUGU C CCAAGGG
473	HCV-9226	9226	CAGCUGG CUGAUGAG X CGAA ACGCGGC	GCCGCGU C CCAGCUG
474	HCV-9238	9238	GCUGGAC CUGAUGAG X CGAA AGUCCAG	CUGGACU U GUCCAGC
475	HCV-9241	9241	CCAGCUG CUGAUGAG X CGAA ACAAGUC	GACUUGU C CAGCUGG
476	HCV-9250	9250	AGCAACG CUGAUGAG X CGAA ACCAGCU	AGCUGGU U CGUUGCU
477	HCV-9251	9251	CAGCAAC CUGAUGAG X CGAA AACCAGC	GCUGGUU C GUUGCUG
478	HCV-9254	9254	AACCAGC CUGAUGAG X CGAA ACGAACC	GGUUCGU U GCUGGUU
479	HCV-9278	9278	UGUGAUA CUGAUGAG X CGAA AUGUCUC	GAGACAU A UAUCACA
480	HCV-9280	9280	GCUGUGA CUGAUGAG X CGAA AUAUGUC	GACAUAU A UCACAGC
481	HCV-9282	9282	AGGCUGU CUGAUGAG X CGAA AUAUAUG	CAUAUAU C ACAGCCU
482	HCV-9292	9292	GGCACGA CUGAUGAG X CGAA ACAGGCU	AGCCUGU C UCGUGCC
483	HCV-9326	9326	GUAGGAG CUGAUGAG X CGAA AGGCACC	GGUGCCU A CUCCUAC
484	HCV-9329	9329	AAAGUAG CUGAUGAG X CGAA AGUAGGC	GCCUACU C CUACUUU
485	HCV-9332	9332	CGGAAAG CUGAUGAG X CGAA AGGAGUA	UACUCCU A CUUUCCG
486	HCV-9335	9335	CUACGGA CUGAUGAG X CGAA AGUAGGA	UCCUACU U UCCGUAG
487	HCV-9336	9336	CCUACGG CUGAUGAG X CGAA AAGUAGG	CCUACUU U CCGUAGG
488	HCV-9337	9337	CCCUACG CUGAUGAG X CGAA AAAGUAG	CUACUUU C CGUAGGG
489	HCV-9341	9341	CUACCCC CUGAUGAG X CGAA ACGGAAA	UUUCCGU A GGGGUAG
490	HCV-9347	9347	AGAUGCC CUGAUGAG X CGAA ACCCCUA	UAGGGGU A GGCAUCU
491	HCV-9353	9353	GCAGGUA CUGAUGAG X CGAA AUGCCUA	UAGGCAU C UACCUGC
492	HCV-9355	9355	GAGCAGG CUGAUGAG X CGAA AGAUGCC	GGCAUCU A CCUGCUC
493	HCV-9362	9362	GGUUGGG CUGAUGAG X CGAA AGCAGGU	ACCUGCU C CCCAACC
494	HCV-9385	9385	GAGUGAU CUGAUGAG X CGAA AGCUCCC	GGGAGCU A AUCACUC
495	HCV-9388	9388	CUGGAGU CUGAUGAG X CGAA AUUAGCU	AGCUAAU C ACUCCAG
496	HCV-9392	9392	UGGCCUG CUGAUGAG X CGAA AGUGAUU	AAUCACU C CAGGCCA
497	HCV-9402	9402	GAUGGCC CUGAUGAG X CGAA AUUGGCC	GGCCAAU A GGCCAUC

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20: 3252). The length of stem II may be 2 base-pairs.

Table VI: Additional HCV Hammerhead (HH) Ribozyme and Target Sequence

Pos.	Ribozyme	Substrate
14	CGCCCCC CUGAUGAG X CGAA AUCGGGG	CCCCGAU U GGGGGCG
34	AGUGAUC CUGAUGAG X CGAA AUGGUGG	CCACCAU A GAUCACU
38	GGGGAGU CUGAUGAG X CGAA AUCUAUG	CAUAGAU C ACUCCCC
42	CACAGGG CUGAUGAG X CGAA AGUGAUC	GAUCACU C CCCUGUG
57	AAGACAG CUGAUGAG X CGAA AGUUCCU	AGGAACU A CUGUCUU
62	GCGUGAA CUGAUGAG X CGAA ACAGUAG	CUACUGU C UUCACGC
64	CUGCGUG CUGAUGAG X CGAA AGACAGU	ACUGUCU U CACGCAG
65	UCUGCGU CUGAUGAG X CGAA AAGACAG	CUGUCUU C ACGCAGA
79	AUGGCUA CUGAUGAG X CGAA ACGCUUU	AAAGCGU C UAGCCAU
81	CCAUGGC CUGAUGAG X CGAA AGACGCU	AGCGUCU A GCCAUGG
92	UCAUACU CUGAUGAG X CGAA ACGCCAU	AUGGCGU U AGUAUGA
93	CUCAUAC CUGAUGAG X CGAA AACGCCA	UGGCGUU A GUAUGAG
96	ACACUCA CUGAUGAG X CGAA ACUAACG	CGUUAGU A UGAGUGU
104	GCUGCAC CUGAUGAG X CGAA ACACUCA	UGAGUGU C GUGCAGC
142	AGACCAC CUGAUGAG X CGAA AUGGCUC	GAGCCAU A GUGGUCU
192	AAGAAAG CUGAUGAG X CGAA ACCCGGU	ACCGGGU C CUUUCUU
195	UCCAAGA CUGAUGAG X CGAA AGGACCC	GGGUCCU U UCUUGGA
196	AUCCAAG CUGAUGAG X CGAA AAGGACC	GGUCCUU U CUUGGAU
197	GAUCCAA CUGAUGAG X CGAA AAAGGAC	GUCCUUU C UUGGAUC
204	GCGGGUU CUGAUGAG X CGAA AUCCAAG	CUUGGAU C AACCCGC
227	ACGCCCA CUGAUGAG X CGAA AUCUCCA	UGGAGAU U UGGGCGU
228	CACGCCC CUGAUGAG X CGAA AAUCUCC	GGAGAUU U GGGCGUG
282	GUACCAC CUGAUGAG X CGAA AGGCCUU	AAGGCCU U GUGGUAC
354	GGUUUAG CUGAUGAG X CGAA AUUCGUG	CACGAAU C CUAAACC
357	UGAGGUU CUGAUGAG X CGAA AGGAUUC	GAAUCCU A AACCUCA
363	UUUCUUU CUGAUGAG X CGAA AGGUUUA	UAAACCU C AAAGAAA
381	UAGGUGU CUGAUGAG X CGAA ACGUUUG	CAAACGU A ACACCUA
388	GCGGCGG CUGAUGAG X CGAA AGGUGUU	AACACCU A CCGCCGC
431	CACCAAC CUGAUGAG X CGAA AUCUGAC	GUCAGAU C GUUGGUG
434	CUCCACC CUGAUGAG X CGAA ACGAUCU	AGAUCGU U GGUGGAG
443	ACACGUA CUGAUGAG X CGAA ACUCCAC	GUGGAGU U UACGUGU
444	AACACGU CUGAUGAG X CGAA AACUCCA	UGGAGUU U ACGUGUU
445	CAACACG CUGAUGAG X CGAA AAACUCC	GGAGUUU A CGUGUUG
451	GCGCGGC CUGAUGAG X CGAA ACACGUA	UACGUGU U GCCGCGC
516	CUUCCAC CUGAUGAG X CGAA AGGUUGC	GCAACCU C GUGGAAG
688	AUUGCGC CUGAUGAG X CGAA ACCUCCG	CGGAGGU C GCGCAAU
702	AUGACCU CUGAUGAG X CGAA ACCCAGA	UCUGGGU A AGGUCAU
719	CGCACGU CUGAUGAG X CGAA AGGGUAU	AUACCCU C ACGUGCG
740	ACCCCAU CUGAUGAG X CGAA AGGUCGG	CCGACCU C AUGGGGU
861	AUAGAGA CUGAUGAG X CGAA AGAGCAA	UUGCUCU U UCUCUAU

862	GAUAGAG CUGAUGAG X CGAA AAGAGCA	UGCUCUU U CUCUAUC
863	AGAUAGA CUGAUGAG X CGAA AAAGAGC	GCUCUUU C UCUAUCU
865	GAAGAUA CUGAUGAG X CGAA AGAAAGA	UCUUUCU C UAUCUUC
867	AGGAAGA CUGAUGAG X CGAA AGAGAAA	UUUCUCU A UCUUCCU
869	AGAGGAA CUGAUGAG X CGAA AUAGAGA	UCUCUAU C UUCCUCU
871	CAAGAGG CUGAUGAG X CGAA AGAUAGA	UCUAUCU U CCUCUUG
872	CCAAGAG CUGAUGAG X CGAA AAGAUAG	CUAUCUU C CUCUUGG
875	GGGCCAA CUGAUGAG X CGAA AGGAAGA	UCUUCCU C UUGGCCC
877	CAGGGCC CUGAUGAG X CGAA AGAGGAA	UUCCUCU U GGCCCUG
889	CAAACAG CUGAUGAG X CGAA ACAGCAG	CUGCUGU C CUGUUUG
894	AUGGUCA CUGAUGAG X CGAA ACAGGAC	GUCCUGU U UGACCAU
895	GAUGGUC CUGAUGAG X CGAA AACAGGA	UCCUGUU U GACCAUC
902	AAGCUGG CUGAUGAG X CGAA AUGGUCA	UGACCAU C CCAGCUU
909	UAAGCGG CUGAUGAG X CGAA AGCUGGG	CCCAGCU U CCGCUUA
910	AUAAGCG CUGAUGAG X CGAA AAGCUGG	CCAGCUU C CGCUUAU
915	ACCUGAU CUGAUGAG X CGAA AGCGGAA	UUCCGCU U AUCAGGU
916	CACCUGA CUGAUGAG X CGAA AAGCGGA	UCCGCUU A UCAGGUG
918	CGCACCU CUGAUGAG X CGAA AUAAGCG	CGCUUAU C AGGUGCG
934	CAGCCCG CUGAUGAG X CGAA AUGCGUU	AACGCAU C CGGGCUG
943	GACAUGG CUGAUGAG X CGAA ACAGCCC	GGGCUGU A CCAUGUC
950	CAUUCGU CUGAUGAG X CGAA ACAUGGU	ACCAUGU C ACGAAUG
964	UGAGUUG CUGAUGAG X CGAA AGCAGUC	GACUGCU C CAACUCA
970	AAUGCUU CUGAUGAG X CGAA AGUUGGA	UCCAACU C AAGCAUU
977	CAUACAC CUGAUGAG X CGAA AUGCUUG	CAAGCAU U GUGUAUG
1008	CCGGGGG CUGAUGAG X CGAA AUGCAUG	CAUGCAU A CCCCCGG
1067	UGGGAGU CUGAUGAG X CGAA AGCGCUA	UAGCGCU C ACUCCCA
1071	AGCGUGG CUGAUGAG X CGAA AGUGAGC	GCUCACU C CCACGCU
1079	UGGCCGC CUGAUGAG X CGAA AGCGUGG	CCACGCU C GCGGCCA
1100	UAGUGGG CUGAUGAG X CGAA AUGCUGG	CCAGCAU C CCCACUA
1107	AUUGUCG CUGAUGAG X CGAA AGUGGGG	CCCCACU A CGACAAU
1115	GGCGUCG CUGAUGAG X CGAA AUUGUCG	CGACAAU A CGACGCC
1152	GAACAGA CUGAUGAG X CGAA AGCGGCC	GGCCGCU U UCUGUUC
1181	AUCCGCA CUGAUGAG X CGAA AGGUCCC	GGGACCU C UGCGGAU
1199	GGGAGAC CUGAUGAG X CGAA AGGAAAA	UUUUCCU C GUCUCCC
1202	ACUGGGA CUGAUGAG X CGAA ACGAGGA	UCCUCGU C UCCCAGU
1204	CAACUGG CUGAUGAG X CGAA AGACGAG	CUCGUCU C CCAGUUG
1210	GGUGAAC CUGAUGAG X CGAA ACUGGGA	UCCCAGU U GUUCACC
1213	GAAGGUG CUGAUGAG X CGAA ACAACUG	CAGUUGU U CACCUUC
1214	AGAAGGU CUGAUGAG X CGAA AACAACU	AGUUGUU C ACCUUCU
1219	AGGCGAG CUGAUGAG X CGAA AGGUGAA	UUCACCU U CUCGCCU
1220	GAGGCGA CUGAUGAG X CGAA AAGGUGA	UCACCUU C UCGCCUC
1222	GCGAGGC CUGAUGAG X CGAA AGAAGGU	ACCUUCU C GCCUCGC
1227	UACCGGC CUGAUGAG X CGAA AGGCGAG	CUCGCCU C GCCGGUA
1234	UGUCUCA CUGAUGAG X CGAA ACCGGCG	CGCCGGU A UGAGACA
1244	AGUCCUG CUGAUGAG X CGAA ACUGUCU	AGACAGU A CAGGACU
1257	AUUGAGC CUGAUGAG X CGAA AUUGCAG	CUGCAAU U GCUCAAU

1261	AUAGAUU CUGAUGAG X CGAA AGCAAUU	AAUUGCU C AAUCUAU
1265	CGGGAUA CUGAUGAG X CGAA AUUGAGC	GCUCAAU C UAUCCCG
1267	GCCGGGA CUGAUGAG X CGAA AGAUUGA	UCAAUCU A UCCCGGC
1269	UGGCCGG CUGAUGAG X CGAA AUAGAUU	AAUCUAU C CCGGCCA
1299	AUAUCCC CUGAUGAG X CGAA AGCCAUG	CAUGGCU U GGGAUAU
1305	AUCAUCA CUGAUGAG X CGAA AUCCCAA	UUGGGAU A UGAUGAU
1321	UGUAGGC CUGAUGAG X CGAA ACCAGUU	AACUGGU C GCCUACA
1326	GCUGUUG CUGAUGAG X CGAA AGGCGAC	GUCGCCU A CAACAGC
1337	ACACCAC CUGAUGAG X CGAA AGGGCUG	CAGCCCU A GUGGUGU
1345	UAACUGC CUGAUGAG X CGAA ACACCAC	GUGGUGU C GCAGUUA
1351	CCGGAGU CUGAUGAG X CGAA ACUGCGA	UCGCAGU U ACUCCGG
1352	UCCGGAG CUGAUGAG X CGAA AACUGCG	CGCAGUU A CUCCGGA
1355	GGAUCCG CUGAUGAG X CGAA AGUAACU	AGUUACU C CGGAUCC
1361	CUUGUGG CUGAUGAG X CGAA AUCCGGA	UCCGGAU C CCACAAG
1449	AAGACCU CUGAUGAG X CGAA AGCCCAG	CUGGGCU A AGGUCUU
1454	CAAUCAA CUGAUGAG X CGAA ACCUUAG	CUAAGGU C UUGAUUG
1456	CACAAUC CUGAUGAG X CGAA AGACCUU	AAGGUCU U GAUUGUG
1460	ACAUCAC CUGAUGAG X CGAA AUCAAGA	UCUUGAU U GUGAUGU
1468	AAAGAGU CUGAUGAG X CGAA ACAUCAC	GUGAUGU U ACUCUUU
1469	CAAAGAG CUGAUGAG X CGAA AACAUCA	UGAUGUU A CUCUUUG
1472	CGGCAAA CUGAUGAG X CGAA AGUAACA	UGUUACU C UUUGCCG
1474	GCCGGCA CUGAUGAG X CGAA AGAGUAA	UUACUCU U UGCCGGC
1475	CGCCGGC CUGAUGAG X CGAA AAGAGUA	UACUCUU U GCCGGCG
1484	CCCCGUC CUGAUGAG X CGAA ACGCCGG	CCGGCGU U GACGGGG
1493	UGUAAGU CUGAUGAG X CGAA ACCCCGU	ACGGGGU C ACUUACA
1497	GUCGUGU CUGAUGAG X CGAA AGUGACC	GGUCACU U ACACGAC
1498	UGUCGUG CUGAUGAG X CGAA AAGUGAC	GUCACUU A CACGACA
1513	AGCUUGC CUGAUGAG X CGAA ACCCCCC	GGGGGGU C GCAAGCU
1521	GUGUGGC CUGAUGAG X CGAA AGCUUGC	GCAAGCU C GCCACAC
1538	AGGACGU CUGAUGAG X CGAA ACGCUCU	AGAGCGU C ACGUCCU
1543	GAAGAAG CUGAUGAG X CGAA ACGUGAC	GUCACGU C CUUCUUC
1546	GGUGAAG CUGAUGAG X CGAA AGGACGU	ACGUCCU U CUUCACC
1547	GGGUGAA CUGAUGAG X CGAA AAGGACG	CGUCCUU C UUCACCC
1549	UUGGGUG CUGAUGAG X CGAA AGAAGGA	UCCUUCU U CACCCAA
1550	CUUGGGU CUGAUGAG X CGAA AAGAAGG	CCUUCUU C ACCCAAG
1574	UGAGCUG CUGAUGAG X CGAA AUUCUCU	AGAGAAU C CAGCUCA
1580	UGUUUAU CUGAUGAG X CGAA AGCUGGA	UCCAGCU C AUAAACA
1583	UGGUGUU CUGAUGAG X CGAA AUGAGCU	AGCUCAU A AACACCA
1607	UCCUGUU CUGAUGAG X CGAA AUGUGCC	GGCACAU C AACAGGA
1636	GUUGAGG CUGAUGAG X CGAA AUUCAUU	AAUGAAU C CCUCAAC
1640	CGGUGUU CUGAUGAG X CGAA AGGGAUU	AAUCCCU C AACACCG
1651	GGCAAAG CUGAUGAG X CGAA ACCCGGU	ACCGGGU U CUUUGCC
1652	CGGCAAA CUGAUGAG X CGAA AACCCGG	CCGGGUU C UUUGCCG
1654	UGCGGCA CUGAUGAG X CGAA AGAACCC	GGGUUCU U UGCCGCA
1655	GUGCGGC CUGAUGAG X CGAA AAGAACC	GGUUCUU U GCCGCAC
1666	UGCGUAG CUGAUGAG X CGAA ACAGUGC	GCACUGU U CUACGCA

1667	GUGCGUA CUGAUGAG X CGAA AACAGUG	CACUGUU C UACGCAC
1669	GUGUGCG CUGAUGAG X CGAA AGAACAG	CUGUUCU A CGCACAC
1681	CGAGUUG CUGAUGAG X CGAA ACUUGUG	CACAAGU U CAACUCG
1682	ACGAGUU CUGAUGAG X CGAA AACUUGU	ACAAGUU C AACUCGU
1687	UCCGGAC CUGAUGAG X CGAA AGUUGAA	UUCAACU C GUCCGGA
1690	GCAUCCG CUGAUGAG X CGAA ACGAGUU	AACUCGU C CGGAUGC
1723	GUCGAUG CUGAUGAG X CGAA AGCUGCA	UGCAGCU C CAUCGAC
1764	GGCUCGG CUGAUGAG X CGAA AUAGGUG	CACCUAU A CCGAGCC
1773	AGGUCCC CUGAUGAG X CGAA AGGCUCG	CGAGCCU A GGGACCU
1785	GGCCUCU CUGAUGAG X CGAA AUCCAGG	CCUGGAU C AGAGGCC
1794	CAGCAGU CUGAUGAG X CGAA AGGCCUC	GAGGCCU U ACUGCUG
1861	GAAACAG CUGAUGAG X CGAA ACACUGG	CCAGUGU A CUGUUUC
1866	GGGGUGA CUGAUGAG X CGAA ACAGUAC	GUACUGU U UCACCCC
1867	UGGGGUG CUGAUGAG X CGAA AACAGUA	UACUGUU U CACCCCA
1868	UUGGGGU CUGAUGAG X CGAA AAACAGU	ACUGUUU C ACCCCAA
1955	UGUUGAG CUGAUGAG X CGAA AGCAGCA	UGCUGCU U CUCAACA
1956	UUGUUGA CUGAUGAG X CGAA AAGCAGC	GCUGCUU C UCAACAA
1958	UGUUGUU CUGAUGAG X CGAA AGAAGCA	UGCUUCU C AACAACA
2020	CUUGGUG CUGAUGAG X CGAA ACCCAGU	ACUGGGU U CACCAAG
2021	UCUUGGU CUGAUGAG X CGAA AACCCAG	CUGGGUU C ACCAAGA
2094	CGAAAGC CUGAUGAG X CGAA AUCCGUG	CACGGAU U GCUUUCG
2098	CUUCCGA CUGAUGAG X CGAA AGCAAUC	GAUUGCU U UCGGAAG
2099	GCUUCCG CUGAUGAG X CGAA AAGCAAU	AUUGCUU U CGGAAGC
2100	UGCUUCC CUGAUGAG X CGAA AAAGCAA	UUGCUUU C GGAAGCA
2157	AUACACC CUGAUGAG X CGAA AGGUGUU	AACACCU A GGUGUAU
2163	UCAACUA CUGAUGAG X CGAA ACACCUA	UAGGUGU A UAGUUGA
2165	AGUCAAC CUGAUGAG X CGAA AUACACC	GGUGUAU A GUUGACU
2168	GGUAGUC CUGAUGAG X CGAA ACUAUAC	GUAUAGU U GACUACC
2173	GUAUGGG CUGAUGAG X CGAA AGUCAAC	GUUGACU A CCCAUAC
2179	GAGCCUG CUGAUGAG X CGAA AUGGGUA	UACCCAU A CAGGCUC
2186	AGUGCCA CUGAUGAG X CGAA AGCCUGU	ACAGGCU C UGGCACU
2194	GCAGGGG CUGAUGAG X CGAA AGUGCCA	UGGCACU A CCCCUGC
2207	UAAAGUU CUGAUGAG X CGAA ACAGUGC	GCACUGU C AACUUUA
2212	GAUGGUA CUGAUGAG X CGAA AGUUGAC	GUCAACU U UACCAUC
2213	AGAUGGU CUGAUGAG X CGAA AAGUUGA	UCAACUU U ACCAUCU
2214	AAGAUGG CUGAUGAG X CGAA AAAGUUG	CAACUUU A CCAUCUU
2222	UAACCUU CUGAUGAG X CGAA AAGAUGG	CCAUCUU U AAGGUUA
2223	CUAACCU CUGAUGAG X CGAA AAAGAUG	CAUCUUU A AGGUUAG
2228	ACAUCCU CUGAUGAG X CGAA ACCUUAA	UUAAGGU U AGGAUGU
2229	UACAUCC CUGAUGAG X CGAA AACCUUA	UAAGGUU A GGAUGUA
2236	CCCCACA CUGAUGAG X CGAA ACAUCCU	AGGAUGU A UGUGGGG
2283	UCUCCUC CUGAUGAG X CGAA AGUCCAG	CUGGACU C GAGGAGA
2366	AACAGGG CUGAUGAG X CGAA AGUGUCU	AGACACU U CCCUGUU
2367	GAACAGG CUGAUGAG X CGAA AAGUGUC	GACACUU C CCUGUUC
2373	GUGAAGG CUGAUGAG X CGAA ACAGGGA	UCCCUGU U CCUUCAC
2374	GGUGAAG CUGAUGAG X CGAA AACAGGG	CCCUGUU C CUUCACC

2377	GGUGGUG CUGAUGAG X CGAA AGGAACA	UGUUCCU U CACCACC
2378	GGGUGGU CUGAUGAG X CGAA AAGGAAC	GUUCCUU C ACCACCC
2387	GAGCCGG CUGAUGAG X CGAA AGGGUGG	CCACCCU A CCGGCUC
2394	GUGGACA CUGAUGAG X CGAA AGCCGGU	ACCGGCU C UGUCCAC
2398	ACCAGUG CUGAUGAG X CGAA ACAGAGC	GCUCUGU C CACUGGU
2406	UGGAUCA CUGAUGAG X CGAA ACCAGUG	CACUGGU U UGAUCCA
2407	GUGGAUC CUGAUGAG X CGAA AACCAGU	ACUGGUU U GAUCCAC
2411	GGAGGUG CUGAUGAG X CGAA AUCAAAC	GUUUGAU C CACCUCC
2443	GUACAGG CUGAUGAG X CGAA ACUGCAC	GUGCAGU A CCUGUAC
2449	UAUACCG CUGAUGAG X CGAA ACAGGUA	UACCUGU A CGGUAUA
2454	GACCCUA CUGAUGAG X CGAA ACCGUAC	GUACGGU A UAGGGUC
2456	CUGACCC CUGAUGAG X CGAA AUACCGU	ACGGUAU A GGGUCAG
2461	AACCGCU CUGAUGAG X CGAA ACCCUAU	AUAGGGU C AGCGGUU
2468	AGGAGAC CUGAUGAG X CGAA ACCGCUG	CAGCGGU U GUCUCCU
2471	CAAAGGA CUGAUGAG X CGAA ACAACCG	CGGUUGU C UCCUUUG
2473	CACAAAG CUGAUGAG X CGAA AGACAAC	GUUGUCU C CUUUGUG
2476	GAUCACA CUGAUGAG X CGAA AGGAGAC	GUCUCCU U UGUGAUC
2477	UGAUCAC CUGAUGAG X CGAA AAGGAGA	UCUCCUU U GUGAUCA
2483	CCCAUUU CUGAUGAG X CGAA AUCACAA	UUGUGAU C AAAUGGG
2494	CACGAUA CUGAUGAG X CGAA ACUCCCA	UGGGAGU A UAUCGUG
2496	AACACGA CUGAUGAG X CGAA AUACUCC	GGAGUAU A UCGUGUU
2498	GCAACAC CUGAUGAG X CGAA AUAUACU	AGUAUAU C GUGUUGC
2503	GAAAAGC CUGAUGAG X CGAA ACACGAU	AUCGUGU U GCUUUUC
2507	GAAGGAA CUGAUGAG X CGAA AGCAACA	UGUUGCU U UUCCUUC
2508	AGAAGGA CUGAUGAG X CGAA AAGCAAC	GUUGCUU U UCCUUCU
2509	GAGAAGG CUGAUGAG X CGAA AAAGCAA	UUGCUUU U CCUUCUC
2510	GGAGAAG CUGAUGAG X CGAA AAAAGCA	UGCUUUU C CUUCUCC
2513	CCAGGAG CUGAUGAG X CGAA AGGAAAA	UUUUCCU U CUCCUGG
2514	GCCAGGA CUGAUGAG X CGAA AAGGAAA	UUUCCUU C UCCUGGC
2516	CCGCCAG CUGAUGAG X CGAA AGAAGGA	UCCUUCU C CUGGCGG
2545	CAUCCAC CUGAUGAG X CGAA AGCAGGC	GCCUGCU U GUGGAUG
2564	CCUGGGC CUGAUGAG X CGAA AUCAGCA	UGCUGAU A GCCCAGG
2614	GGCCAGG CUGAUGAG X CGAA ACGCCGC	GCGGCGU C CCUGGCC
2636	AGGAGAG CUGAUGAG X CGAA AUGCCAU	AUGGCAU U CUCUCCU
2637	AAGGAGA CUGAUGAG X CGAA AAUGCCA	UGGCAUU C UCUCCUU
2639	GGAAGGA CUGAUGAG X CGAA AGAAUGC	GCAUUCU C UCCUUCC
2641	AAGGAAG CUGAUGAG X CGAA AGAGAAU	AUUCUCU C CUUCCUU
2644	CACAAGG CUGAUGAG X CGAA AGGAGAG	CUCUCCU U CCUUGUG
2645	ACACAAG CUGAUGAG X CGAA AAGGAGA	UCUCCUU C CUUGUGU
2648	AAAACAC CUGAUGAG X CGAA AGGAAGG	CCUUCCU U GUGUUUU
2653	ACAGAAA CUGAUGAG X CGAA ACACAAG	CUUGUGU U UUUCUGU
2654	CACAGAA CUGAUGAG X CGAA AACACAA	UUGUGUU U UUCUGUG
2655	GCACAGA CUGAUGAG X CGAA AAACACA	UGUGUUU U UCUGUGC
2656	GGCACAG CUGAUGAG X CGAA AAAACAC	GUGUUUU U CUGUGCC
2657	CGGCACA CUGAUGAG X CGAA AAAAACA	UGUUUUU C UGUGCCG
2732	GGAGCAG CUGAUGAG X CGAA AGCAGCG	CGCUGCU C CUGCUCC

2749	UGGUGGU CUGAUGAG X CGAA ACGCCAG	CUGGCGU U ACCACCA
2750	GUGGUGG CUGAUGAG X CGAA AACGCCA	UGGCGUU A CCACCAC
2791	UCCACAC CUGAUGAG X CGAA AUGCAGC	GCUGCAU C GUGUGGA
2807	CUACAAA CUGAUGAG X CGAA ACCACCC	GGGUGGU U UUUGUAG
2808	CCUACAA CUGAUGAG X CGAA AACCACC	GGUGGUU U UUGUAGG
2809	ACCUACA CUGAUGAG X CGAA AAACCAC	GUGGUUU U UGUAGGU
2810	GACCUAC CUGAUGAG X CGAA AAAACCA	UGGUUUU U GUAGGUC
2813	UUAGACC CUGAUGAG X CGAA ACAAAAA	UUUUUGU A GGUCUAA
2817	AGUAUUA CUGAUGAG X CGAA ACCUACA	UGUAGGU C UAAUACU
2819	AGAGUAU CUGAUGAG X CGAA AGACCUA	UAGGUCU A AUACUCU
2822	UCAAGAG CUGAUGAG X CGAA AUUAGAC	GUCUAAU A CUCUUGA
2825	AGGUCAA CUGAUGAG X CGAA AGUAUUA	UAAUACU C UUGACCU
2827	CAAGGUC CUGAUGAG X CGAA AGAGUAU	AUACUCU U GACCUUG
2833	UGGUGAC CUGAUGAG X CGAA AGGUCAA	UUGACCU U GUCACCA
2836	GUGUGGU CUGAUGAG X CGAA ACAAGGU	ACCUUGU C ACCACAC
2845	CACUUUG CUGAUGAG X CGAA AGUGUGG	CCACACU A CAAAGUG
2854	GGCGAGG CUGAUGAG X CGAA ACACUUU	AAAGUGU U CCUCGCC
2855	UGGCGAG CUGAUGAG X CGAA AACACUU	AAGUGUU C CUCGCCA
2858	GCCUGGC CUGAUGAG X CGAA AGGAACA	UGUUCCU C GCCAGGC
2867	ACCAUAU CUGAUGAG X CGAA AGCCUGG	CCAGGCU C AUAUGGU
2870	ACCACCA CUGAUGAG X CGAA AUGAGCC	GGCUCAU A UGGUGGU
2889	CUGGUGA CUGAUGAG X CGAA AAAGUAU	AUACUUU A UCACCAG
2891	CCCUGGU CUGAUGAG X CGAA AUAAAGU	ACUUUAU C ACCAGGG
2993	CAAAGAU CUGAUGAG X CGAA AGCUCUG	CAGAGCU A AUCUUUG
2996	UGUCAAA CUGAUGAG X CGAA AUUAGCU	AGCUAAU C UUUGACA
2998	AAUGUCA CUGAUGAG X CGAA AGAUUAG	CUAAUCU U UGACAUU
2999	UAAUGUC CUGAUGAG X CGAA AAGAUUA	UAAUCUU U GACAUUA
3005	GUUUGGU CUGAUGAG X CGAA AUGUCAA	UUGACAU U ACCAAAC
3006	AGUUUGG CUGAUGAG X CGAA AAUGUCA	UGACAUU A CCAAACU
3014	CGAGCAG CUGAUGAG X CGAA AGUUUGG	CCAAACU C CUGCUCG
3020	GAAUGGC CUGAUGAG X CGAA AGCAGGA	UCCUGCU C GCCAUUC
3026	GACCGAG CUGAUGAG X CGAA AUGGCGA	UCGCCAU U CUCGGUC
3027	GGACCGA CUGAUGAG X CGAA AAUGGCG	CGCCAUU C UCGGUCC
3029	GCGGACC CUGAUGAG X CGAA AGAAUGG	CCAUUCU C GGUCCGC
3033	AUGAGCG CUGAUGAG X CGAA ACCGAGA	UCUCGGU C CGCUCAU
3038	GCACCAU CUGAUGAG X CGAA AGCGGAC	GUCCGCU C AUGGUGC
3047	CAGCCUG CUGAUGAG X CGAA AGCACCA	UGGUGCU C CAGGCUG
3073	UACAAAG CUGAUGAG X CGAA ACGGCAU	AUGCCGU A CUUUGUA
3076	GCGUACA CUGAUGAG X CGAA AGUACGG	CCGUACU U UGUACGC
3077	CGCGUAC CUGAUGAG X CGAA AAGUACG	CGUACUU U GUACGCG
3080	GAGCGCG CUGAUGAG X CGAA ACAAAGU	ACUUUGU A CGCGCUC
3087	AGCCCCU CUGAUGAG X CGAA AGCGCGU	ACGCGCU C AGGGGCU
3095	CACGAAU CUGAUGAG X CGAA AGCCCCU	AGGGGCU U AUUCGUG
3096	GCACGAA CUGAUGAG X CGAA AAGCCCC	GGGGCUU A UUCGUGC
3098	AUGCACG CUGAUGAG X CGAA AUAAGCC	GGCUUAU U CGUGCAU
3099	CAUGCAC CUGAUGAG X CGAA AAUAAGC	GCUUAUU C GUGCAUG

3112	CCGCACC CUGAUGAG X CGAA ACAUGCA	UGCAUGU U GGUGCGG
3125	CUCCGGC CUGAUGAG X CGAA ACUUUCC	GGAAAGU A GCCGGAG
3180	ACGUACG CUGAUGAG X CGAA ACCUGUC	GACAGGU A CGUACGU
3184	AUAGACG CUGAUGAG X CGAA ACGUACC	GGUACGU A CGUCUAU
3188	GGUCAUA CUGAUGAG X CGAA ACGUACG	CGUACGU C UAUGACC
3190	AUGGUCA CUGAUGAG X CGAA AGACGUA	UACGUCU A UGACCAU
3198	GGGGUAA CUGAUGAG X CGAA AUGGUCA	UGACCAU C UUACCCC
3200	GCGGGGU CUGAUGAG X CGAA AGAUGGU	ACCAUCU U ACCCCGC
3201	AGCGGGG CUGAUGAG X CGAA AAGAUGG	CCAUCUU A CCCCGCU
3254	CGGGCUC CUGAUGAG X CGAA ACUGCCA	UGGCAGU A GAGCCCG
3269	UGUCAGA CUGAUGAG X CGAA AAGACGA	UCGUCUU C UCUGACA
3271	CAUGUCA CUGAUGAG X CGAA AGAAGAC	GUCUUCU C UGACAUG
3374	GUCCCAG CUGAUGAG X CGAA AGUAUCU	AGAUACU U CUGGGAC
3375	GGUCCCA CUGAUGAG X CGAA AAGUAUC	GAUACUU C UGGGACC
3390	UCAAUGC CUGAUGAG X CGAA AUCGGCC	GGCCGAU A GCAUUGA
3395	GCCCUUC CUGAUGAG X CGAA AUGCUAU	AUAGCAU U GAAGGGC
3436	UUGGGCG CUGAUGAG X CGAA AGGCCGU	ACGGCCU A CGCCCAA
3458	AACCAAG CUGAUGAG X CGAA AGGCCCC	GGGGCCU A CUUGGUU
3461	UGCAACC CUGAUGAG X CGAA AGUAGGC	GCCUACU U GGUUGCA
3465	ACAAUGC CUGAUGAG X CGAA ACCAAGU	ACUUGGU U GCAUUGU
3470	UAGUAAC CUGAUGAG X CGAA AUGCAAC	GUUGCAU U GUUACUA
3473	GGCUAGU CUGAUGAG X CGAA ACAAUGC	GCAUUGU U ACUAGCC
3474	AGGCUAG CUGAUGAG X CGAA AACAAUG	CAUUGUU A CUAGCCU
3477	GUGAGGC CUGAUGAG X CGAA AGUAACA	UGUUACU A GCCUCAC
3506	CCCCUUC CUGAUGAG X CGAA ACCUGGU	ACCAGGU C GAAGGGG
3544	CAGGAAA CUGAUGAG X CGAA AUUGUGU	ACACAAU C UUUCCUG
3546	GCCAGGA CUGAUGAG X CGAA AGAUUGU	ACAAUCU U UCCUGGC
3547	CGCCAGG CUGAUGAG X CGAA AAGAUUG	CAAUCUU U CCUGGCG
3548	UCGCCAG CUGAUGAG X CGAA AAAGAUU	AAUCUUU C CUGGCGA
3563	CACCAUU CUGAUGAG X CGAA ACGCAGG	CCUGCGU U AAUGGUG
3564	ACACCAU CUGAUGAG X CGAA AACGCAG	CUGCGUU A AUGGUGU
3584	CGUGGAA CUGAUGAG X CGAA ACGGUCC	GGACCGU C UUCCACG
3586	GCCGUGG CUGAUGAG X CGAA AGACGGU	ACCGUCU U CCACGGC
3587	CGCCGUG CUGAUGAG X CGAA AAGACGG	CCGUCUU C CACGGCG
3632	UUUGGGU CUGAUGAG X CGAA AUUGGGC	GCCCAAU C ACCCAAA
3643	AUUAGUG CUGAUGAG X CGAA ACAUUUG	CAAAUGU A CACUAAU
3648	UCUACAU CUGAUGAG X CGAA AGUGUAC	GUACACU A AUGUAGA
3653	CUUGGUC CUGAUGAG X CGAA ACAUUAG	CUAAUGU A GACCAAG
3665	AGCCGAC CUGAUGAG X CGAA AGGUCUU	AAGACCU C GUCGGCU
3668	GCCAGCC CUGAUGAG X CGAA ACGAGGU	ACCUCGU C GGCUGGC
3720	UCCGAGC CUGAUGAG X CGAA ACCGCAG	CUGCGGU A GCUCGGA
3758	CCGGAAU CUGAUGAG X CGAA ACGUCAG	CUGACGU C AUUCCGG
3815	AAUAGGA CUGAUGAG X CGAA ACGGGUC	GACCCGU C UCCUAUU
3817	CAAAUAG CUGAUGAG X CGAA AGACGGG	CCCGUCU C CUAUUUG
3820	CUUCAAA CUGAUGAG X CGAA AGGAGAC	GUCUCCU A UUUGAAG
3822	CCCUUCA CUGAUGAG X CGAA AUAGGAG	CUCCUAU U UGAAGGG

3823	GCCCUUC CUGAUGAG X CGAA AAUAGGA	UCCUAUU U GAAGGGC
3832	ACCCGAA CUGAUGAG X CGAA AGCCCUU	AAGGGCU C UUCGGGU
3834	CCACCCG CUGAUGAG X CGAA AGAGCCC	GGGCUCU U CGGGUGG
3925	GGGUAUG CUGAUGAG X CGAA AGUCCAC	GUGGACU U CAUACCC
3926	CGGGUAU CUGAUGAG X CGAA AAGUCCA	UGGACUU C AUACCCG
3929	CAACGGG CUGAUGAG X CGAA AUGAAGU	ACUUCAU A CCCGUUG
3935	UAGACUC CUGAUGAG X CGAA ACGGGUA	UACCCGU U GAGUCUA
3940	UUCCAUA CUGAUGAG X CGAA ACUCAAC	GUUGAGU C UAUGGAA
3942	GUUUCCA CUGAUGAG X CGAA AGACUCA	UGAGUCU A UGGAAAC
3951	CGCAUAG CUGAUGAG X CGAA AGUUUCC	GGAAACU A CUAUGCG
3954	GACCGCA CUGAUGAG X CGAA AGUAGUU	AACUACU A UGCGGUC
3961	GACCGGG CUGAUGAG X CGAA ACCGCAU	AUGCGGU C CCCGGUC
3968	CCGUGAA CUGAUGAG X CGAA ACCGGGG	CCCCGGU C UUCACGG
3970	GUCCGUG CUGAUGAG X CGAA AGACCGG	CCGGUCU U CACGGAC
3971	UGUCCGU CUGAUGAG X CGAA AAGACCG	CGGUCUU C ACGGACA
3982	GGGAGAU CUGAUGAG X CGAA AGUUGUC	GACAACU C AUCUCCC
3985	CGGGGGA CUGAUGAG X CGAA AUGAGUU	AACUCAU C UCCCCCG
3987	GCCGGGG CUGAUGAG X CGAA AGAUGAG	CUCAUCU C CCCCGGC
3998	UCUGCGG CUGAUGAG X CGAA ACGGCCG	CGGCCGU A CCGCAGA
4009	CACUUGG CUGAUGAG X CGAA AUGUCUG	CAGACAU U CCAAGUG
4010	CCACUUG CUGAUGAG X CGAA AAUGUCU	AGACAUU C CAAGUGG
4023	GCGUGUA CUGAUGAG X CGAA AUGGGCC	GGCCCAU C UACACGC
4025	GAGCGUG CUGAUGAG X CGAA AGAUGGG	CCCAUCU A CACGCUC
4032	CCAGUGG CUGAUGAG X CGAA AGCGUGU	ACACGCU C CCACUGG
4094	GGACGAG CUGAUGAG X CGAA ACCUUGU	ACAAGGU A CUCGUCC
4097	UCAGGAC CUGAUGAG X CGAA AGUACCU	AGGUACU C GUCCUGA
4100	GGUUCAG CUGAUGAG X CGAA ACGAGUA	UACUCGU C CUGAACC
4111	GGCAACA CUGAUGAG X CGAA AUGGGUU	AACCCAU C UGUUGCC
4126	AAAACCC CUGAUGAG X CGAA AGGUGGC	GCCACCU U GGGUUUU
4131	GCCCCAA CUGAUGAG X CGAA ACCCAAG	CUUGGGU U UUGGGGC
4132	CGCCCCA CUGAUGAG X CGAA AACCCAA	UUGGGUU U UGGGGCG
4133	ACGCCCC CUGAUGAG X CGAA AAACCCA	UGGGUUU U GGGGCGU
4141	AGACAUA CUGAUGAG X CGAA ACGCCCC	GGGGCGU A UAUGUCU
4143	UUAGACA CUGAUGAG X CGAA AUACGCC	GGCGUAU A UGUCUAA
4147	UGCCUUA CUGAUGAG X CGAA ACAUAUA	UAUAUGU C UAAGGCA
4149	UGUGCCU CUGAUGAG X CGAA AGACAUA	UAUGUCU A AGGCACA
4161	GGGUCGG CUGAUGAG X CGAA ACCAUGU	ACAUGGU A CCGACCC
4196	CCGUGGU CUGAUGAG X CGAA AUGGUCC	GGACCAU U ACCACGG
4197	CCCGUGG CUGAUGAG X CGAA AAUGGUC	GACCAUU A CCACGGG
4214	AGUACGU CUGAUGAG X CGAA AUGGGGG	CCCCCAU C ACGUACU
4219	GGUGGAG CUGAUGAG X CGAA ACGUGAU	AUCACGU A CUCCACC
4222	AUAGGUG CUGAUGAG X CGAA AGUACGU	ACGUACU C CACCUAU
4257	CCCCCAG CUGAUGAG X CGAA ACAUCCA	UGGAUGU U CUGGGGG
4258	GCCCCCA CUGAUGAG X CGAA AACAUCC	GGAUGUU C UGGGGGC
4270	GAUAUCA CUGAUGAG X CGAA AGGCGCC	GGCGCCU A UGAUAUC
4275	AUUAUGA CUGAUGAG X CGAA AUCAUAG	CUAUGAU A UCAUAAU

4277	AUAUUAU CUGAUGAG X CGAA AUAUCAU	AUGAUAU C AUAAUAU
4300	GUCAGUU CUGAUGAG X CGAA AGUGGCA	UGCCACU C AACUGAC
4309	GGUAGUC CUGAUGAG X CGAA AGUCAGU	ACUGACU C GACUACC
4314	AGGAUGG CUGAUGAG X CGAA AGUCGAG	CUCGACU A CCAUCCU
4319	UGCCCAG CUGAUGAG X CGAA AUGGUAG	CUACCAU C CUGGGCA
4328	CUGUGCC CUGAUGAG X CGAA AUGCCCA	UGGGCAU C GGCACAG
4389	GGAGGCG CUGAUGAG X CGAA AGCGGUG	CACCGCU A CGCCUCC
4395	GAUCCCG CUGAUGAG X CGAA AGGCGUA	UACGCCU C CGGGAUC
4402	GGUAACC CUGAUGAG X CGAA AUCCCGG	CCGGGAU C GGUUACC
4406	GCACGGU CUGAUGAG X CGAA ACCGAUC	GAUCGGU U ACCGUGC
4407	GGCACGG CUGAUGAG X CGAA AACCGAU	AUCGGUU A CCGUGCC
4427	CCUCCUC CUGAUGAG X CGAA AUAUUUG	CAAAUAU U GAGGAGG
4440	UUGGACA CUGAUGAG X CGAA AGCCACC	GGUGGCU C UGUCCAA
4465	GCCAUAG CUGAUGAG X CGAA AGGGGAU	AUCCCCU U CUAUGGC
4466	UGCCAUA CUGAUGAG X CGAA AAGGGGA	UCCCCUU C UAUGGCA
4468	CUUGCCA CUGAUGAG X CGAA AGAAGGG	CCCUUCU A UGGCAAG
4512	AAAAUGA CUGAUGAG X CGAA AUGCCUU	AAGGCAU C UCAUUUU
4514	AGAAAAU CUGAUGAG X CGAA AGAUGCC	GGCAUCU C AUUUUCU
4517	GGCAGAA CUGAUGAG X CGAA AUGAGAU	AUCUCAU U UUCUGCC
4518	UGGCAGA CUGAUGAG X CGAA AAUGAGA	UCUCAUU U UCUGCCA
4519	GUGGCAG CUGAUGAG X CGAA AAAUGAG	CUCAUUU U CUGCCAC
4520	AGUGGCA CUGAUGAG X CGAA AAAAUGA	UCAUUUU C UGCCACU
4550	UUGCGGC CUGAUGAG X CGAA AGCUCAU	AUGAGCU C GCCGCAA
4564	GAGGCCU CUGAUGAG X CGAA ACAGCUU	AAGCUGU C AGGCCUC
4571	UGAUUCC CUGAUGAG X CGAA AGGCCUG	CAGGCCU C GGAAUCA
4602	ACGUCAA CUGAUGAG X CGAA ACCCCGG	CCGGGGU C UUGACGU
4604	ACACGUC CUGAUGAG X CGAA AGACCCC	GGGGUCU U GACGUGU
4612	UAUGACG CUGAUGAG X CGAA ACACGUC	GACGUGU C CGUCAUA
4637	CGAUAAC CUGAUGAG X CGAA ACAUCUC	GAGAUGU C GUUAUCG
4640	CCACGAU CUGAUGAG X CGAA ACGACAU	AUGUCGU U AUCGUGG
4641	GCCACGA CUGAUGAG X CGAA AACGACA	UGUCGUU A UCGUGGC
4643	UUGCCAC CUGAUGAG X CGAA AUAACGA	UCGUUAU C GUGGCAA
4659	GUCAUUA CUGAUGAG X CGAA AGCGUCU	AGACGCU C UAAUGAC
4661	CCGUCAU CUGAUGAG X CGAA AGAGCGU	ACGCUCU A AUGACGG
4684	CGAGUCA CUGAUGAG X CGAA AGUCACC	GGUGACU U UGACUCG
4685	CCGAGUC CUGAUGAG X CGAA AAGUCAC	GUGACUU U GACUCGG
4690	GAUCACC CUGAUGAG X CGAA AGUCAAA	UUUGACU C GGUGAUC
4715	UCUGGGU CUGAUGAG X CGAA ACACAUG	CAUGUGU C ACCCAGA
4727	UGAAAUC CUGAUGAG X CGAA ACUGUCU	AGACAGU C GAUUUCA
4731	AAGCUGA CUGAUGAG X CGAA AUCGACU	AGUCGAU U UCAGCUU
4732	CAAGCUG CUGAUGAG X CGAA AAUCGAC	GUCGAUU U CAGCUUG
4733	CCAAGCU CUGAUGAG X CGAA AAAUCGA	UCGAUUU C AGCUUGG
4738	GGGAUCC CUGAUGAG X CGAA AGCUGAA	UUCAGCU U GGAUCCC
4743	AAGGUGG CUGAUGAG X CGAA AUCCAAG	CUUGGAU C CCACCUU
4750	AAUGGUA CUGAUGAG X CGAA AGGUGGG	CCCACCU U UACCAUU
4751	CAAUGGU CUGAUGAG X CGAA AAGGUGG	CCACCUU U ACCAUUG

4752	UCAAUGG CUGAUGAG X CGAA AAAGGUG	CACCUUU A CCAUUGA
4757	UCGUCUC CUGAUGAG X CGAA AUGGUAA	UUACCAU U GAGACGA
4824	CCUCCCC CUGAUGAG X CGAA ACCCCUG	CAGGGGU A GGGGAGG
4835	ACCUGUA CUGAUGAG X CGAA AUGCCUC	GAGGCAU C UACAGGU
4837	AAACCUG CUGAUGAG X CGAA AGAUGCC	GGCAUCU A CAGGUUU
4843	AGUCACA CUGAUGAG X CGAA ACCUGUA	UACAGGU U UGUGACU
4844	GAGUCAC CUGAUGAG X CGAA AACCUGU	ACAGGUU U GUGACUC
4851	UCUCCCG CUGAUGAG X CGAA AGUCACA	UGUGACU C CGGGAGA
4867	CAUGCCC CUGAUGAG X CGAA AGGGCCG	CGGCCCU C GGGCAUG
4876	AGAAUCG CUGAUGAG X CGAA ACAUGCC	GGCAUGU U CGAUUCU
4877	AAGAAUC CUGAUGAG X CGAA AACAUGC	GCAUGUU C GAUUCUU
4881	ACCGAAG CUGAUGAG X CGAA AUCGAAC	GUUCGAU U CUUCGGU
4882	GACCGAA CUGAUGAG X CGAA AAUCGAA	UUCGAUU C UUCGGUC
4884	AGGACCG CUGAUGAG X CGAA AGAAUCG	CGAUUCU U CGGUCCU
4885	CAGGACC CUGAUGAG X CGAA AAGAAUC	GAUUCUU C GGUCCUG
4889	CACACAG CUGAUGAG X CGAA ACCGAAG	CUUCGGU C CUGUGUG
4903	CGCGUCA CUGAUGAG X CGAA AGCACUC	GAGUGCU A UGACGCG
5011	UUCCCAG CUGAUGAG X CGAA ACUCCAG	CUGGAGU U CUGGGAA
5012	UUUCCCA CUGAUGAG X CGAA AACUCCA	UGGAGUU C UGGGAAA
5024	CUGUGAA CUGAUGAG X CGAA ACGCUUU	AAAGCGU C UUCACAG
5026	GCCUGUG CUGAUGAG X CGAA AGACGCU	AGCGUCU U CACAGGC
5027	GGCCUGU CUGAUGAG X CGAA AAGACGC	GCGUCUU C ACAGGCC
5036	UGUGGGU CUGAUGAG X CGAA AGGCCUG	CAGGCCU C ACCCACA
5045	GGGCAUC CUGAUGAG X CGAA AUGUGGG	CCCACAU A GAUGCCC
5056	GGACAGG CUGAUGAG X CGAA AGUGGGC	GCCCACU U CCUGUCC
5057	GGGACAG CUGAUGAG X CGAA AAGUGGG	CCCACUU C CUGUCCC
5062	GGUUUGG CUGAUGAG X CGAA ACAGGAA	UUCCUGU C CCAAACC
5089	GUAAGGG CUGAUGAG X CGAA AGUUGUC	GACAACU U CCCUUAC
5090	GGUAAGG CUGAUGAG X CGAA AAGUUGU	ACAACUU C CCUUACC
5094	ACCAGGU CUGAUGAG X CGAA AGGGAAG	CUUCCCU U ACCUGGU
5095	UACCAGG CUGAUGAG X CGAA AAGGGAA	UUCCCUU A CCUGGUA
5139	GGAGGUG CUGAUGAG X CGAA AGCCUGA	UCAGGCU C CACCUCC
5145	CACGAUG CUGAUGAG X CGAA AGGUGGA	UCCACCU C CAUCGUG
5149	AUCCCAC CUGAUGAG X CGAA AUGGAGG	CCUCCAU C GUGGGAU
5157	CACAUUU CUGAUGAG X CGAA AUCCCAC	GUGGGAU C AAAUGUG
5172	CGUAUGA CUGAUGAG X CGAA ACACUUC	GAAGUGU C UCAUACG
5174	GCCGUAU CUGAUGAG X CGAA AGACACU	AGUGUCU C AUACGGC
5177	UAAGCCG CUGAUGAG X CGAA AUGAGAC	GUCUCAU A CGGCUUA
5183	UAGGUUU CUGAUGAG X CGAA AGCCGUA	UACGGCU U AAACCUA
5184	GUAGGUU CUGAUGAG X CGAA AAGCCGU	ACGGCUU A AACCUAC
5190	UGCAGCG CUGAUGAG X CGAA AGGUUUA	UAAACCU A CGCUGCA
5225	CGGCUCC CUGAUGAG X CGAA AGCCUAU	AUAGGCU A GGAGCCG
5234	CAUUUUG CUGAUGAG X CGAA ACGGCUC	GAGCCGU U CAAAAUG
5235	UCAUUUU CUGAUGAG X CGAA AACGGCU	AGCCGUU C AAAAUGA
5246	UGAGGGU CUGAUGAG X CGAA AUCUCAU	AUGAGAU C ACCCUCA
5252	GAUGUGU CUGAUGAG X CGAA AGGGUGA	UCACCCU C ACACAUC

5259	GUUAUGG CUGAUGAG X CGAA AUGUGUG	CACACAU C CCAUAAC
5264	AUUUGGU CUGAUGAG X CGAA AUGGGAU	AUCCCAU A ACCAAAU
5272	CAUGAUG CUGAUGAG X CGAA AUUUGGU	ACCAAAU U CAUCAUG
5273	CCAUGAU CUGAUGAG X CGAA AAUUUGG	CCAAAUU C AUCAUGG
5276	AUGCCAU CUGAUGAG X CGAA AUGAAUU	AAUUCAU C AUGGCAU
5290	GUCGGCC CUGAUGAG X CGAA ACAUGCA	UGCAUGU C GGCCGAC
5349	GCGGCCA CUGAUGAG X CGAA AGCUGCA	UGCAGCU C UGGCCGC
5384	CCACAAU CUGAUGAG X CGAA ACCACAC	GUGUGGU C AUUGUGG
5387	UACCCAC CUGAUGAG X CGAA AUGACCA	UGGUCAU U GUGGGUA
5394	AUGAUCC CUGAUGAG X CGAA ACCCACA	UGUGGGU A GGAUCAU
5402	CGGACAA CUGAUGAG X CGAA AUGAUCC	GGAUCAU U UUGUCCG
5403	CCGGACA CUGAUGAG X CGAA AAUGAUC	GAUCAUU U UGUCCGG
5404	CCCGGAC CUGAUGAG X CGAA AAAUGAU	AUCAUUU U GUCCGGG
5407	CCUCCCG CUGAUGAG X CGAA ACAAAAU	AUUUUGU C CGGGAGG
5441	GGUAGAG CUGAUGAG X CGAA ACUUCCC	GGGAAGU C CUCUACC
5444	CCCGGUA CUGAUGAG X CGAA AGGACUU	AAGUCCU C UACCGGG
5446	CUCCCGG CUGAUGAG X CGAA AGAGGAC	GUCCUCU A CCGGGAG
5455	UUCAUCG CUGAUGAG X CGAA ACUCCCG	CGGGAGU U CGAUGAA
5456	UUUCAUC CUGAUGAG X CGAA AACUCCC	GGGAGUU C GAUGAAA
5479	GAGGUGU CUGAUGAG X CGAA AGGCGCA	UGCGCCU C ACACCUC
5486	UGUAAGG CUGAUGAG X CGAA AGGUGUG	CACACCU C CCUUACA
5490	UCGAUGU CUGAUGAG X CGAA AGGGAGG	CCUCCCU U ACAUCGA
5491	UUCGAUG CUGAUGAG X CGAA AAGGGAG	CUCCCUU A CAUCGAA
5495	CCUGUUC CUGAUGAG X CGAA AUGUAAG	CUUACAU C GAACAGG
5513	GCUCGGC CUGAUGAG X CGAA AGCUGCA	UGCAGCU C GCCGAGC
5540	GCAACCC CUGAUGAG X CGAA AGUGCCU	AGGCACU C GGGUUGC
5545	UUGCAGC CUGAUGAG X CGAA ACCCGAG	CUCGGGU U GCUGCAA
5644	GCUGAUG CUGAUGAG X CGAA AGUUCCA	UGGAACU U CAUCAGC
5645	CGCUGAU CUGAUGAG X CGAA AAGUUCC	GGAACUU C AUCAGCG
5648	UCCCGCU CUGAUGAG X CGAA AUGAAGU	ACUUCAU C AGCGGGA
5657	AAUACUG CUGAUGAG X CGAA AUGCCGC	GCGGGAU A CAGUAUU
5662	UGCUAAA CUGAUGAG X CGAA ACUGUAU	AUACAGU A UUUAGCA
5664	CCUGCUA CUGAUGAG X CGAA AUACUGU	ACAGUAU U UAGCAGG
5665	GCCUGCU CUGAUGAG X CGAA AAUACUG	CAGUAUU U AGCAGGC
5666	AGCCUGC CUGAUGAG X CGAA AAAUACU	AGUAUUU A GCAGGCU
5677	CAGAGUG CUGAUGAG X CGAA AUAAGCC	GGCUUAU C CACUCUG
5682	CCAGGCA CUGAUGAG X CGAA AGUGGAU	AUCCACU C UGCCUGG
5702	GUGAUGC CUGAUGAG X CGAA AUCGCGG	CCGCGAU A GCAUCAC
5707	CAUCAGU CUGAUGAG X CGAA AUGCUAU	AUAGCAU C ACUGAUG
5719	GGCUGUG CUGAUGAG X CGAA AUGCCAU	AUGGCAU U CACAGCC
5720	AGGCUGU CUGAUGAG X CGAA AAUGCCA	UGGCAUU C ACAGCCU
5728	GGUGAUA CUGAUGAG X CGAA AGGCUGU	ACAGCCU C UAUCACC
5730	CUGGUGA CUGAUGAG X CGAA AGAGGCU	AGCCUCU A UCACCAG
5732	GACUGGU CUGAUGAG X CGAA AUAGAGG	CCUCUAU C ACCAGUC
5739	GUGAGCG CUGAUGAG X CGAA ACUGGUG	CACCAGU C CGCUCAC
5744	GGGUGGU CUGAUGAG X CGAA AGCGGAC	GUCCGCU C ACCACCC

5757	AGGAGGG CUGAUGAG X CGAA AUUCUGG	CCAGAAU A CCCUCCU
5762	UGAACAG CUGAUGAG X CGAA AGGGUAU	AUACCCU C CUGUUCA
5774	CCCCUAA CUGAUGAG X CGAA AUGUUGA	UCAACAU C UUAGGGG
5776	UCCCCCU CUGAUGAG X CGAA AGAUGUU	AACAUCU U AGGGGGA
5777	AUCCCCC CUGAUGAG X CGAA AAGAUGU	ACAUCUU A GGGGGAU
5796	GCGAGUU CUGAUGAG X CGAA AGCAGCC	GGCUGCU C AACUCGC
5808	GCACUGG CUGAUGAG X CGAA AGGAGCG	CGCUCCU C CCAGUGC
5820	AAGGCCG CUGAUGAG X CGAA AGCAGCA	UGCUGCU U CGGCCUU
5885	UGUCCAC CUGAUGAG X CGAA AGCACCU	AGGUGCU U GUGGACA
5894	CCGCCAG CUGAUGAG X CGAA AUGUCCA	UGGACAU U CUGGCGG
5895	CCCGCCA CUGAUGAG X CGAA AAUGUCC	GGACAUU C UGGCGGG
5986	AGGGAGC CUGAUGAG X CGAA AGUUAAC	GUUAACU U GCUCCCU
5999	GGGAGAG CUGAUGAG X CGAA AUGGCAG	CUGCCAU C CUCUCCC
6002	CGGGGGA CUGAUGAG X CGAA AGGAUGG	CCAUCCU C UCCCCCG
6101	CGAACGC CUGAUGAG X CGAA AUCAGCC	GGCUGAU A GCGUUCG
6112	ACCCCGC CUGAUGAG X CGAA AAGCGAA	UUCGCUU C GCGGGGU
6120	ACGUGGU CUGAUGAG X CGAA ACCCCGC	GCGGGGU A ACCACGU
6128	UGGGGGA CUGAUGAG X CGAA ACGUGGU	ACCACGU U UCCCCCA
6129	GUGGGGG CUGAUGAG X CGAA AACGUGG	CCACGUU U CCCCCAC
6130	CGUGGGG CUGAUGAG X CGAA AAACGUG	CACGUUU C CCCCACG
6142	AGGCACG CUGAUGAG X CGAA AGUGCGU	ACGCACU A CGUGCCU
6173	UCUGAGU CUGAUGAG X CGAA ACACGUG	CACGUGU A ACUCAGA
6177	AGGAUCU CUGAUGAG X CGAA AGUUACA	UGUAACU C AGAUCCU
6182	UGGAGAG CUGAUGAG X CGAA AUCUGAG	CUCAGAU C CUCUCCA
6185	GGCUGGA CUGAUGAG X CGAA AGGAUCU	AGAUCCU C UCCAGCC
6187	GAGGCUG CUGAUGAG X CGAA AGAGGAU	AUCCUCU C CAGCCUC
6194	UGAUGGU CUGAUGAG X CGAA AGGCUGG	CCAGCCU C ACCAUCA
6200	GCUGAGU CUGAUGAG X CGAA AUGGUGA	UCACCAU C ACUCAGC
6204	AGCAGCU CUGAUGAG X CGAA AGUGAUG	CAUCACU C AGCUGCU
6221	ACUGGUG CUGAUGAG X CGAA AGCCUCU	AGAGGCU U CACCAGU
6222	CACUGGU CUGAUGAG X CGAA AAGCCUC	GAGGCUU C ACCAGUG
6233	CCUCAUU CUGAUGAG X CGAA AUCCACU	AGUGGAU U AAUGAGG
6234	UCCUCAU CUGAUGAG X CGAA AAUCCAC	GUGGAUU A AUGAGGA
6247	UGGCGUG CUGAUGAG X CGAA AGCAGUC	GACUGCU C CACGCCA
6259	CGAGCCG CUGAUGAG X CGAA AGCAUGG	CCAUGCU C CGGCUCG
6265	UAGCCAC CUGAUGAG X CGAA AGCCGGA	UCCGGCU C GUGGCUA
6272	CAUCCUU CUGAUGAG X CGAA AGCCACG	CGUGGCU A AAGGAUG
6281	AGUCCCA CUGAUGAG X CGAA ACAUCCU	AGGAUGU U UGGGACU
6282	CAGUCCC CUGAUGAG X CGAA AACAUCC	GGAUGUU U GGGACUG
6293	CCGUGCA CUGAUGAG X CGAA AUCCAGU	ACUGGAU A UGCACGG
6304	GUCAGUC CUGAUGAG X CGAA ACACCGU	ACGGUGU U GACUGAC
6313	GGUCUUG CUGAUGAG X CGAA AGUCAGU	ACUGACU U CAAGACC
6314	AGGUCUU CUGAUGAG X CGAA AAGUCAG	CUGACUU C AAGACCU
6326	UGGACUG CUGAUGAG X CGAA AGCCAGG	CCUGGCU C CAGUCCA
6331	GAGCUUG CUGAUGAG X CGAA ACUGGAG	CUCCAGU C CAAGCUC
6338	UCGGCAG CUGAUGAG X CGAA AGCUUGG	CCAAGCU C CUGCCGA

6349	UCCCGGC CUGAUGAG X CGAA AUUUCGG	CCGAAAU U GCCGGGA
6359	AGAAAGG CUGAUGAG X CGAA ACUCCCG	CGGGAGU C CCUUUCU
6363	GAGAAGA CUGAUGAG X CGAA AGGGACU	AGUCCCU U UCUUCUC
6364	UGAGAAG CUGAUGAG X CGAA AAGGGAC	GUCCCUU U CUUCUCA
6365	AUGAGAA CUGAUGAG X CGAA AAAGGGA	UCCCUUU C UUCUCAU
6367	GCAUGAG CUGAUGAG X CGAA AGAAAGG	CCUUUCU U CUCAUGC
6368	GGCAUGA CUGAUGAG X CGAA AAGAAAG	CUUUCUU C UCAUGCC
6370	UUGGCAU CUGAUGAG X CGAA AGAAGAA	UUCUUCU C AUGCCAA
6385	UCCCUUG CUGAUGAG X CGAA ACCCGCG	CGCGGGU A CAAGGGA
6395	CCCGCCA CUGAUGAG X CGAA ACUCCCU	AGGGAGU C UGGCGGG
6446	GUCCGGU CUGAUGAG X CGAA AUUUGUG	CACAAAU U ACCGGAC
6447	UGUCCGG CUGAUGAG X CGAA AAUUUGU	ACAAAUU A CCGGACA
6458	CGUUUUU CUGAUGAG X CGAA ACAUGUC	GACAUGU C AAAAACG
6468	CUCAUGG CUGAUGAG X CGAA ACCGUUU	AAACGGU U CCAUGAG
6469	CCUCAUG CUGAUGAG X CGAA AACCGUU	AACGGUU C CAUGAGG
6479	GCCCAAC CUGAUGAG X CGAA AUCCUCA	UGAGGAU C GUUGGGC
6482	UAGGCCC CUGAUGAG X CGAA ACGAUCC	GGAUCGU U GGGCCUA
6489	CAGGUUU CUGAUGAG X CGAA AGGCCCA	UGGGCCU A AAACCUG
6520	GAUGGGG CUGAUGAG X CGAA ACGUUCC	GGAACGU U CCCCAUC
6521	UGAUGGG CUGAUGAG X CGAA AACGUUC	GAACGUU C CCCAUCA
6527	ACGCGUU CUGAUGAG X CGAA AUGGGGA	UCCCCAU C AACGCGU
6535	UGUGGUG CUGAUGAG X CGAA ACGCGUU	AACGCGU A CACCACA
6559	CGCCGGG CUGAUGAG X CGAA AGGGUGU	ACACCCU C CCCGGCG
6610	CUCCACG CUGAUGAG X CGAA ACUCUUC	GAAGAGU A CGUGGAG
6620	CCCGCGU CUGAUGAG X CGAA AUCUCCA	UGGAGAU U ACGCGGG
6621	ACCCGCG CUGAUGAG X CGAA AAUCUCC	GGAGAUU A CGCGGGU
6654	GUGGUCA CUGAUGAG X CGAA ACCCGUC	GACGGGU A UGACCAC
6689	GGGCCGG CUGAUGAG X CGAA ACCUGGC	GCCAGGU C CCGGCCC
6781	GACCUGG CUGAUGAG X CGAA AUGUGAC	GUCACAU U CCAGGUC
6854	UGGAAGU CUGAUGAG X CGAA AGCACUG	CAGUGCU C ACUUCCA
6858	AGCAUGG CUGAUGAG X CGAA AGUGAGC	GCUCACU U CCAUGCU
6859	GAGCAUG CUGAUGAG X CGAA AAGUGAG	CUCACUU C CAUGCUC
6866	GGUCGGU CUGAUGAG X CGAA AGCAUGG	CCAUGCU C ACCGACC
6877	AAUGUGG CUGAUGAG X CGAA AGGGGUC	GACCCCU C CCACAUU
6884	CUGCUGU CUGAUGAG X CGAA AUGUGGG	CCCACAU U ACAGCAG
6885	UCUGCUG CUGAUGAG X CGAA AAUGUGG	CCACAUU A CAGCAGA
6900	CUACGUU CUGAUGAG X CGAA AGCCGUC	GACGGCU A AACGUAG
6945	CUAGCUG CUGAUGAG X CGAA AGAGCUG	CAGCUCU U CAGCUAG
6946	GCUAGCU CUGAUGAG X CGAA AAGAGCU	AGCUCUU C AGCUAGC
6951	AAUUGGC CUGAUGAG X CGAA AGCUGAA	UUCAGCU A GCCAAUU
6969	UUCAAGG CUGAUGAG X CGAA AGGCGCA	UGCGCCU U CCUUGAA
6970	CUUCAAG CUGAUGAG X CGAA AAGGCGC	GCGCCUU C CUUGAAG
6973	UGCCUUC CUGAUGAG X CGAA AGGAAGG	CCUUCCU U GAAGGCA
6990	UGGUGGG CUGAUGAG X CGAA AGUGCAU	AUGCACU A CCCACCA
7003	GUCCGGG CUGAUGAG X CGAA AGUCAUG	CAUGACU C CCCGGAC
7019	CCUCGAU CUGAUGAG X CGAA AGGUCAG	CUGACCU C AUCGAGG

7022	UGGCCUC CUGAUGAG X CGAA AUGAGGU	ACCUCAU C GAGGCCA
7064	CACGGGU CUGAUGAG X CGAA AUGUUUC	GAAACAU C ACCCGUG
7078	AUUCUCU CUGAUGAG X CGAA ACUCCAC	GUGGAGU C AGAGAAU
7086	ACCACCU CUGAUGAG X CGAA AUUCUCU	AGAGAAU A AGGUGGU
7094	CCAAAAU CUGAUGAG X CGAA ACCACCU	AGGUGGU A AUUUUGG
7097	AGUCCAA CUGAUGAG X CGAA AUUACCA	UGGUAAU U UUGGACU
7098	GAGUCCA CUGAUGAG X CGAA AAUUACC	GGUAAUU U UGGACUC
7099	AGAGUCC CUGAUGAG X CGAA AAAUUAC	GUAAUUU U GGACUCU
7105	GUCGAAA CUGAUGAG X CGAA AGUCCAA	UUGGACU C UUUCGAC
7107	GGGUCGA CUGAUGAG X CGAA AGAGUCC	GGACUCU U UCGACCC
7108	CGGGUCG CUGAUGAG X CGAA AAGAGUC	GACUCUU U CGACCCG
7109	GCGGGUC CUGAUGAG X CGAA AAAGAGU	ACUCUUU C GACCCGC
7147	UGCAACG CUGAUGAG X CGAA AUACUUC	GAAGUAU C CGUUGCA
7151	CUGCUGC CUGAUGAG X CGAA ACGGAUA	UAUCCGU U GCAGCAG
7163	UUCGCAG CUGAUGAG X CGAA AUCUCUG	CAGAGAU C CUGCGAA
7174	CUUCUUG CUGAUGAG X CGAA AUUUUCG	CGAAAAU C CAAGAAG
7183	GGGGGGG CUGAUGAG X CGAA ACUUCUU	AAGAAGU U CCCCCCC
7184	CGGGGGG CUGAUGAG X CGAA AACUUCU	AGAAGUU C CCCCCCG
7227	AACAGUG CUGAUGAG X CGAA AGGGUUG	CAACCCU C CACUGUU
7240	UUUCCAG CUGAUGAG X CGAA ACUCUAA	UUAGAGU C CUGGAAA
7308	GGUAUUG CUGAUGAG X CGAA AGGGCCC	GGGCCCU C CAAUACC
7313	GAGGCGG CUGAUGAG X CGAA AUUGGAG	CUCCAAU A CCGCCUC
7320	UUCCGUG CUGAUGAG X CGAA AGGCGGU	ACCGCCU C CACGGAA
7340	UCAGAAC CUGAUGAG X CGAA ACCGUCC	GGACGGU U GUUCUGA
7343	CUGUCAG CUGAUGAG X CGAA ACAACCG	CGGUUGU U CUGACAG
7344	UCUGUCA CUGAUGAG X CGAA AACAACC	GGUUGUU C UGACAGA
7363	GGCAGAA CUGAUGAG X CGAA ACACGGU	ACCGUGU C UUCUGCC
7365	AAGGCAG CUGAUGAG X CGAA AGACACG	CGUGUCU U CUGCCUU
7366	CAAGGCA CUGAUGAG X CGAA AAGACAC	GUGUCUU C UGCCUUG
7372	CUCCGCC CUGAUGAG X CGAA AGGCAGA	UCUGCCU U GGCGGAG
7405	CGAUCCG CUGAUGAG X CGAA AGGUGGC	GGGAGCU C CGGAUCG
7446	UGAUCGG CUGAUGAG X CGAA AGGGGCG	CGCCCCU C CCGAUCA
7452	GAGGUCU CUGAUGAG X CGAA AUCGGGA	UCCCGAU C AGACCUC
7459	GUCGUCA CUGAUGAG X CGAA AGGUCUG	CAGACCU C UGACGAC
7480	AACGUCA CUGAUGAG X CGAA AUUCUUU	AAAGAAU C UGACGUU
7487	ACGACUC CUGAUGAG X CGAA ACGUCAG	CUGACGU U GAGUCGU
7492	GGAGUAC CUGAUGAG X CGAA ACUCAAC	GUUGAGU C GUACUCC
7495	GGAGGAG CUGAUGAG X CGAA ACGACUC	GAGUCGU A CUCCUCC
7609	CCAUGUG CUGAUGAG X CGAA AGGACAU	AUGUCCU A CACAUGG
7631	AUGGCGU CUGAUGAG X CGAA AUCAGGG	CCCUGAU C ACGCCAU
7675	GUUGCUC CUGAUGAG X CGAA ACGCGUU	AACGCGU U GAGCAAC
7684	CAGCAGA CUGAUGAG X CGAA AGUUGCU	AGCAACU C UCUGCUG
7686	CGCAGCA CUGAUGAG X CGAA AGAGUUG	CAACUCU C UGCUGCG
7695	UUGUGGU CUGAUGAG X CGAA ACGCAGC	GCUGCGU C ACCACAA
7709	UGGCAUA CUGAUGAG X CGAA ACCAUGU	ACAUGGU C UAUGCCA
7711	UGUGGCA CUGAUGAG X CGAA AGACCAU	AUGGUCU A UGCCACA

7754	CAAAGGU CUGAUGAG X CGAA ACCUUCU	AGAAGGU C ACCUUUG
7759	UCUGUCA CUGAUGAG X CGAA AGGUGAC	GUCACCU U UGACAGA
7760	GUCUGUC CUGAUGAG X CGAA AAGGUGA	UCACCUU U GACAGAC
7802	UCUCCUU CUGAUGAG X CGAA AGCACGU	ACGUGCU C AAGGAGA
7825	AACUGUG CUGAUGAG X CGAA ACGCCUU	AAGGCGU C CACAGUU
7832	UAGCCUU CUGAUGAG X CGAA ACUGUGG	CCACAGU U AAGGCUA
7833	UUAGCCU CUGAUGAG X CGAA AACUGUG	CACAGUU A AGGCUAA
7844	CGGAUAG CUGAUGAG X CGAA AGUUUAG	CUAAACU U CUAUCCG
7845	ACGGAUA CUGAUGAG X CGAA AAGUUUA	UAAACUU C UAUCCGU
7884	UUGGCCG CUGAUGAG X CGAA AUGUGGG	CCCACAU U CGGCCAA
7885	UUUGGCC CUGAUGAG X CGAA AAUGUGG	CCACAUU C GGCCAAA
7922	GGUUCCG CUGAUGAG X CGAA ACGUCCU	AGGACGU C CGGAACC
7931	UGCUGGA CUGAUGAG X CGAA AGGUUCC	GGAACCU A UCCAGCA
7933	CUUGCUG CUGAUGAG X CGAA AUAGGUU	AACCUAU C CAGCAAG
7946	UGUGGUU CUGAUGAG X CGAA AUGGCCU	AGGCCAU U AACCACA
7947	AUGUGGU CUGAUGAG X CGAA AAUGGCC	GGCCAUU A ACCACAU
8000	UGGUGUC CUGAUGAG X CGAA AUUGGUG	CACCAAU U GACACCA
8012	UUGCCAU CUGAUGAG X CGAA AUGGUGG	CCACCAU C AUGGCAA
8030	CGCAGAA CUGAUGAG X CGAA ACUUCAC	GUGAAGU U UUCUGCG
8031	ACGCAGA CUGAUGAG X CGAA AACUUCA	UGAAGUU U UCUGCGU
8032	GACGCAG CUGAUGAG X CGAA AAACUUC	GAAGUUU U CUGCGUC
8033	GGACGCA CUGAUGAG X CGAA AAAACUU	AAGUUUU C UGCGUCC
8039	CCGGUUG CUGAUGAG X CGAA ACGCAGA	UCUGCGU C CAACCGG
8070	AUAAGGC CUGAUGAG X CGAA AGCUGGC	GCCAGCU C GCCUUAU
8081	CUGGGAA CUGAUGAG X CGAA ACGAUAA	UUAUCGU A UUCCCAG
8083	GUCUGGG CUGAUGAG X CGAA AUACGAU	AUCGUAU U CCCAGAC
8084	GGUCUGG CUGAUGAG X CGAA AAUACGA	UCGUAUU C CCAGACC
8099	AUACACG CUGAUGAG X CGAA ACUCCCA	UGGGAGU U CGUGUAU
8100	CAUACAC CUGAUGAG X CGAA AACUCCC	GGGAGUU C GUGUAUG
8105	UCUCGCA CUGAUGAG X CGAA ACACGAA	UUCGUGU A UGCGAGA
8121	UCGUAAA CUGAUGAG X CGAA AGCCAUU	AAUGGCU C UUUACGA
8123	CGUCGUA CUGAUGAG X CGAA AGAGCCA	UGGCUCU U UACGACG
8124	ACGUCGU CUGAUGAG X CGAA AAGAGCC	GGCUCUU U ACGACGU
8125	CACGUCG CUGAUGAG X CGAA AAAGAGC	GCUCUUU A CGACGUG
8135	GGGUGGA CUGAUGAG X CGAA ACCACGU	ACGUGGU C UCCACCC
8137	AAGGGUG CUGAUGAG X CGAA AGACCAC	GUGGUCU C CACCCUU
8144	CCUGAGG CUGAUGAG X CGAA AGGGUGG	CCACCCU U CCUCAGG
8145	GCCUGAG CUGAUGAG X CGAA AAGGGUG	CACCCUU C CUCAGGC
8148	ACGGCCU CUGAUGAG X CGAA AGGAAGG	CCUUCCU C AGGCCGU
8164	GUACGAG CUGAUGAG X CGAA AGCCCAU	AUGGGCU C CUCGUAC
8167	UCCGUAC CUGAUGAG X CGAA AGGAGCC	GGCUCCU C GUACGGA
8177	AGUACUG CUGAUGAG X CGAA AAUCCGU	ACGGAUU C CAGUACU
8185	CCCAGGA CUGAUGAG X CGAA AGUACUG	CAGUACU C UCCUGGG
8241	AAGCCCA CUGAUGAG X CGAA AGGGCUU	AAGCCCU A UGGGCUU
8248	AUACGAG CUGAUGAG X CGAA AGCCCAU	AUGGGCU U CUCGUAU
8249	CAUACGA CUGAUGAG X CGAA AAGCCCA	UGGGCUU C UCGUAUG

8251	GUCAUAC CUGAUGAG X CGAA AGAAGCC	GGCUUCU C GUAUGAC
8254	GGUGUCA CUGAUGAG X CGAA ACGAGAA	UUCUCGU A UGACACC
8269	UGAGUCA CUGAUGAG X CGAA AGCAGCG	CGCUGCU U UGACUCA
8270	UUGAGUC CUGAUGAG X CGAA AAGCAGC	GCUGCUU U GACUCAA
8275	GACUGUU CUGAUGAG X CGAA AGUCAAA	UUUGACU C AACAGUC
8282	UCUCAGU CUGAUGAG X CGAA ACUGUUG	CAACAGU C ACUGAGA
8297	CAACACG CUGAUGAG X CGAA AUGUCGC	GCGACAU C CGUGUUG
8303	ACUCCUC CUGAUGAG X CGAA ACACGGA	UCCGUGU U GAGGAGU
8311	GUAGAUU CUGAUGAG X CGAA ACUCCUC	GAGGAGU C AAUCUAC
8315	AUUGGUA CUGAUGAG X CGAA AUUGACU	AGUCAAU C UACCAAU
8317	ACAUUGG CUGAUGAG X CGAA AGAUUGA	UCAAUCU A CCAAUGU
8325	AAGUCAC CUGAUGAG X CGAA ACAUUGG	CCAAUGU U GUGACUU
8332	GGGGGCC CUGAUGAG X CGAA AGUCACA	UGUGACU U GGCCCCC
8400	UUUGAAU CUGAUGAG X CGAA AGUCAGG	CCUGACU A AUUCAAA
8403	CCUUUUG CUGAUGAG X CGAA AUUAGUC	GACUAAU U CAAAAGG
8404	CCCUUUU CUGAUGAG X CGAA AAUUAGU	ACUAAUU C AAAAGGG
8472	GUGAGGG CUGAUGAG X CGAA AUUGCCG	CGGCAAU A CCCUCAC
8477	AGCAUGU CUGAUGAG X CGAA AGGGUAU	AUACCCU C ACAUGCU
8485	UUUCAAG CUGAUGAG X CGAA AGCAUGU	ACAUGCU A CUUGAAA
8488	GGCUUUC CUGAUGAG X CGAA AGUAGCA	UGCUACU U GAAAGCC
8565	UCACAGA CUGAUGAG X CGAA AACGACA	UGUCGUU A UCUGUGA
8567	UUUCACA CUGAUGAG X CGAA AUAACGA	UCGUUAU C UGUGAAA
8606	AGACUCG CUGAUGAG X CGAA AGGCUCG	CGAGCCU A CGAGUCU
8612	CCGUGAA CUGAUGAG X CGAA ACUCGUA	UACGAGU C UUCACGG
8614	CUCCGUG CUGAUGAG X CGAA AGACUCG	CGAGUCU U CACGGAG
8615	CCUCCGU CUGAUGAG X CGAA AAGACUC	GAGUCUU C ACGGAGG
8625	CUAGUCA CUGAUGAG X CGAA AGCCUCC	GGAGGCU A UGACUAG
8631	GAGUACC CUGAUGAG X CGAA AGUCAUA	UAUGACU A GGUACUC
8635	GGCAGAG CUGAUGAG X CGAA ACCUAGU	ACUAGGU A CUCUGCC
8677	CAACUCC CUGAUGAG X CGAA AGUCGUA	UACGACU U GGAGUUG
8683	UGUUAUC CUGAUGAG X CGAA ACUCCAA	UUGGAGU U GAUAACA
8687	AUGAUGU CUGAUGAG X CGAA AUCAACU	AGUUGAU A ACAUCAU
8692	GGAGCAU CUGAUGAG X CGAA AUGUUAU	AUAACAU C AUGCUCC
8710	CGCGACC CUGAUGAG X CGAA ACACGUU	AACGUGU C GGUCGCG
8714	CGUGCGC CUGAUGAG X CGAA ACCGACA	UGUCGGU C GCGCACG
8743	GAGGUAG CUGAUGAG X CGAA ACACUCU	AGAGUGU A CUACCUC
8746	AGUGAGG CUGAUGAG X CGAA AGUACAC	GUGUACU A CCUCACU
8750	CACGAGU CUGAUGAG X CGAA AGGUAGU	ACUACCU C ACUCGUG
8754	GGAUCAC CUGAUGAG X CGAA AGUGAGG	CCUCACU C GUGAUCC
8760	GUGGUGG CUGAUGAG X CGAA AUCACGA	UCGUGAU C CCACCAC
8799	GUGUGUC CUGAUGAG X CGAA AGCUGUC	GACAGCU A GACACAC
8808	UUGACUG CUGAUGAG X CGAA AGUGUGU	ACACACU C CAGUCAA
8813	AGGAGUU CUGAUGAG X CGAA ACUGGAG	CUCCAGU C AACUCCU
8818	UAGCCAG CUGAUGAG X CGAA AGUUGAC	GUCAACU C CUGGCUA
8825	UGUUGCC CUGAUGAG X CGAA AGCCAGG	CCUGGCU A GGCAACA
8834	ACAUGAU CUGAUGAG X CGAA AUGUUGC	GCAACAU C AUCAUGU

8837	CAUACAU CUGAUGAG X CGAA AUGAUGU	ACAUCAU C AUGUAUG
8870	UCAUCAA CUGAUGAG X CGAA AUCAUCC	GGAUGAU U UUGAUGA
8872	AGUCAUC CUGAUGAG X CGAA AAAUCAU	AUGAUUU U GAUGACU
8884	GGAGAAG CUGAUGAG X CGAA AGUGAGU	ACUCACU U CUUCUCC
8885	UGGAGAA CUGAUGAG X CGAA AAGUGAG	CUCACUU C UUCUCCA
8887	GAUGGAG CUGAUGAG X CGAA AGAAGUG	CACUUCU U CUCCAUC
8888	GGAUGGA CUGAUGAG X CGAA AAGAAGU	ACUUCUU C UCCAUCC
8890	AAGGAUG CUGAUGAG X CGAA AGAAGAA	UUCUUCU C CAUCCUU
8894	CUAGAAG CUGAUGAG X CGAA AUGGAGA	UCUCCAU C CUUCUAG
8897	GGGCUAG CUGAUGAG X CGAA AGGAUGG	CCAUCCU U CUAGCCC
8898	UGGGCUA CUGAUGAG X CGAA AAGGAUG	CAUCCUU C UAGCCCA
8900	CCUGGGC CUGAUGAG X CGAA AGAAGGA	UCCUUCU A GCCCAGG
8915	CCUUUUC CUGAUGAG X CGAA AGCUGUU	AACAGCU U GAAAAGG
8952	AUGGAGU CUGAUGAG X CGAA ACAGGCC	GGCCUGU U ACUCCAU
8953	AAUGGAG CUGAUGAG X CGAA AACAGGC	GCCUGUU A CUCCAUU
8956	CUCAAUG CUGAUGAG X CGAA AGUAACA	UGUUACU C CAUUGAG
8960	GUGGCUC CUGAUGAG X CGAA AUGGAGU	ACUCCAU U GAGCCAC
8969	GUAGGUC CUGAUGAG X CGAA AGUGGCU	AGCCACU U GACCUAC
8975	UCUGAGG CUGAUGAG X CGAA AGGUCAA	UUGACCU A CCUCAGA
8979	AUGAUCU CUGAUGAG X CGAA AGGUAGG	CCUACCU C AGAUCAU
8984	GUUGAAU CUGAUGAG X CGAA AUCUGAG	CUCAGAU C AUUCAAC
8987	GUCGUUG CUGAUGAG X CGAA AUGAUCU	AGAUCAU U CAACGAC
8988	AGUCGUU CUGAUGAG X CGAA AAUGAUC	GAUCAUU C AACGACU
8996	GACCAUG CUGAUGAG X CGAA AGUCGUU	AACGACU C CAUGGUC
9003	GCGCUAA CUGAUGAG X CGAA ACCAUGG	CCAUGGU C UUAGCGC
9005	AUGCGCU CUGAUGAG X CGAA AGACCAU	AUGGUCU U AGCGCAU
9006	AAUGCGC CUGAUGAG X CGAA AAGACCA	UGGUCUU A GCGCAUU
9013	GAGUGAG CUGAUGAG X CGAA AUGCGCU	AGCGCAU U CUCACUC
9014	GGAGUGA CUGAUGAG X CGAA AAUGCGC	GCGCAUU C UCACUCC
9016	AUGGAGU CUGAUGAG X CGAA AGAAUGC	GCAUUCU C ACUCCAU
9020	AACUAUG CUGAUGAG X CGAA AGUGAGA	UCUCACU C CAUAGUU
9024	GAGUAAC CUGAUGAG X CGAA AUGGAGU	ACUCCAU A GUUACUC
9027	GGAGAGU CUGAUGAG X CGAA ACUAUGG	CCAUAGU U ACUCUCC
9028	UGGAGAG CUGAUGAG X CGAA AACUAUG	CAUAGUU A CUCUCCA
9031	ACCUGGA CUGAUGAG X CGAA AGUAACU	AGUUACU C UCCAGGU
9033	UCACCUG CUGAUGAG X CGAA AGAGUAA	UUACUCU C CAGGUGA
9044	CCCUAUU CUGAUGAG X CGAA AUCUCAC	GUGAGAU C AAUAGGG
9048	GCCACCC CUGAUGAG X CGAA AUUGAUC	GAUCAAU A GGGUGGC
9057	AGGCAUG CUGAUGAG X CGAA AGCCACC	GGUGGCU U CAUGCCU
9058	GAGGCAU CUGAUGAG X CGAA AAGCCAC	GUGGCUU C AUGCCUC
9105	CUGGCCC CUGAUGAG X CGAA AUGUCUC	GAGACAU C GGGCCAG
9169	GAAGAGG CUGAUGAG X CGAA ACUUGCC	GGCAAGU A CCUCUUC
9173	AGUUGAA CUGAUGAG X CGAA AGGUACU	AGUACCU C UUCAACU
9175	CCAGUUG CUGAUGAG X CGAA AGAGGUA	UACCUCU U CAACUGG
9176	CCCAGUU CUGAUGAG X CGAA AAGAGGU	ACCUCUU C AACUGGG
9188	UGGUCCU CUGAUGAG X CGAA ACUGCCC	GGGCAGU A AGGACCA

9200	UGAGUUU CUGAUGAG X CGAA AGCUUGG	CCAAGCU C AAACUCA
9206	UUGGAGU CUGAUGAG X CGAA AGUUUGA	UCAAACU C ACUCCAA
9210	GGGAUUG CUGAUGAG X CGAA AGUGAGU	ACUCACU C CAAUCCC
9215	CGGCCGG CUGAUGAG X CGAA AUUGGAG	CUCCAAU C CCGGCCG
9261	CCGCUGU CUGAUGAG X CGAA ACCAGCA	UGCUGGU U ACAGCGG
9262	CCCGCUG CUGAUGAG X CGAA AACCAGC	GCUGGUU A CAGCGGG
9294	CGGGCAC CUGAUGAG X CGAA AGACAGG	CCUGUCU C GUGCCCG
9313	CCACAUA CUGAUGAG X CGAA ACCAGCG	CGCUGGU U UAUGUGG
9314	ACCACAU CUGAUGAG X CGAA AACCAGC	GCUGGUU U AUGUGGU
9315	CACCACA CUGAUGAG X CGAA AAACCAG	CUGGUUU A UGUGGUG
9409	AAAAGGG CUGAUGAG X CGAA AUGGCCU	AGGCCAU C CCCUUUU
9414	AAAAAAA CUGAUGAG X CGAA AGGGGAU	AUCCCCU U UUUUUUU

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20: 3252). The length of stem II may be 2 base-pairs.

Table VII: HCV Hairpin (HP) Ribozyme and Target Sequence

Pos.	Ribozyme Sequence	Substrate
10	CCCCCA AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC CGAU UGGGGG
59	CGUGAA AGAA GUAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUAC UGUC UUCACG
109	CCUGGA AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC AGCC UCCAGG
209	GCAUUG AGAA GGUU ACCAGAGAAACA X GUACAUUACCUGGUA	AACC CGCU CAAUGC
290	CUAUCA AGAA GUAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUAC UGCC UGAUAG
390	GUGGGC AGAA GUAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUAC CGCC GCCCAC
393	CCUGUG AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC CGCC CACAGG
427	CCAACG AGAA GACC ACCAGAGAAACA X GUACAUUACCUGGUA	GGUC AGAU CGUUGG
505	GGUUGC AGAA GUUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAAC GGUC GCAACC
549	CCUCGG AGAA GCGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCGC CGAC CCGAGG
574	UACCCA AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC AGCC UGGGUA
645	GCCGGG AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC GGCU CCCGGC
652	CAACUA AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC GGCC UAGUUG
671	CCGGGG AGAA GUGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCAC GGAC CCCCGG
726	CGGCGA AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC GGCU UCGCCG
734	CAUGAG AGAA GCGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCGC CGAC CUCAUG
754	CCGACG AGAA GAAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUUC CGCU CGUCGG
852	AAGAGC AGAA GGGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCCC GGUU GCUCUU
883	CAGGAC AGAA GGGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCCC UGCU GUCCUG
886	AAACAG AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC UGUC CUGUUU
891	UGGUCA AGAA GGAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUCC UGUU UGACCA
905	AGCGGA AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC AGCU UCCGCU
911	CUGAUA AGAA GAAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUUC CGCU UAUCAG
960	AGUUGG AGAA GUCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGAC UGCU CCAACU
1050	CCCAAC AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC CGUU GUUGGG
1145	GAAAGC AGAA GCCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGGC GGCC GCUUUC
1148	ACAGAA AGAA GCCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGGC CGCU UUCUGU
1155	UGGCGG AGAA GAAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUUC UGUU CCGCCA
1185	AAACGG AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC GGAU CCGUUU
1190	GAGGAA AGAA GAUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAUC CGUU UUCCUC
1207	GUGAAC AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC AGUU GUUCAC
1331	CACUAG AGAA GUUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAAC AGCC CUAGUG
1357	UGUGGG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC GGAU CCCACA
1370	AUCCAC AGAA GCUU ACCAGAGAAACA X GUACAUUACCUGGUA	AAGC UGUC GUGGAU
1562	UCUCUG AGAA GGCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGCC GGCC CAGAGA
1576	UUUAUG AGAA GGAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUCC AGCU CAUAAA
1596	UGUGCC AGAA GCCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGGC AGCU GGCACA
1616	GUUCAG AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC UGCC CUGAAC
1663	GCGUAG AGAA GUGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCAC UGUU CUACGC
1692	CUGGGC AGAA GGAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUCC GGAU GCCCAG
1713	AGCUGC AGAA GGCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGCC AGCU GCAGCU

1719	CGAUGG AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC AGCU CCAUCG
1797	AAUGCC AGAA GUAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUAC UGCU GGCAUU
1863	GGGUGA AGAA GUAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUAC UGUU UCACCC
1880	CACUAC AGAA GGGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCCC UGUU GUAGUG
1898	GGACCG AGAA GUCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGAC CGAU CGGUCC
1903	GCACCG AGAA GAUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAUC GGUC CGGUGC
1943	CAGCAC AGAA GUCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGAC AGAU GUGCUG
1951	UUGAGA AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC UGCU UCUCAA
1969	UGUGGC AGAA GCGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACGC GGCC GCCACA
2082	CCGUGG AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC UGCC CCACGG
2090	AAAGCA AGAA GUGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCAC GGAU UGCUUU
2316	GCUCCG AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC AGAU CGGAGC
2328	GCAGCG AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC AGCC CGCUGC
2332	AGCAGC AGAA GGCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGCC CGCU GCUGCU
2335	GACAGC AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC UGCU GCUGUC
2338	GUGGAC AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC UGCU GUCCAC
2341	GUCGUG AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC UGUC CACGAC
2370	UGAAGG AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC UGUU CCUUCA
2390	GGACAG AGAA GGUA ACCAGAGAAACA X GUACAUUACCUGGUA	UACC GGCU CUGUCC
2395	CCAGUG AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC UGUC CACUGG
2465	GGAGAC AGAA GCUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAGC GGUU GUCUCC
2522	GCGCGC AGAA GCCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGGC GGAC GCGCGC
2541	UCCACA AGAA GGCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGCC UGCU UGUGGA
2557	GCUAUC AGAA GCAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUGC UGCU GAUAGC
2579	CUCUAG AGAA GCCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGGC CGCC CUAGAG
2627	AAUGCC AGAA GCUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAGC GGAU GGCAUU
2663	GUACCA AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC CGCC UGGUAC
2725	AGGAGC AGAA GCCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGGC CGCU GCUCCU
2728	AGCAGG AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC UGCU CCUGCU
2734	AGCAGG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC UGCU CCUGCU
2740	AACGCC AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC UGCU GGCGUU
2978	UGGGUG AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC GGCC CACCCA
3016	AUGGCG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC UGCU CGCCAU
3030	UGAGCG AGAA GAGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCUC GGUC CGCUCA
3034	ACCAUG AGAA GACC ACCAGAGAAACA X GUACAUUACCUGGUA	GGUC CGCU CAUGGU
3260	GAAGAC AGAA GGCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGCC CGUC GUCUUC
3340	GAGACG AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC UGCC CGUCUC
3344	GGCGGA AGAA GGCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGCC CGUC UCCGCC
3350	CCUUCG AGAA GAGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCUC CGCC CGAAGG
3383	GCUAUC AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC GGCC GAUAGC
3431	GGCGUA AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC GGCC UACGCC
3581	GUGGAA AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC CGUC UUCCAC
3597	UCUUUG AGAA GGCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGCC GGCU CAAAGA
3615	CUUUUG AGAA GGCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGCC GGCC CAAAAG
3669	CAUGCC AGAA GACG ACCAGAGAAACA X GUACAUUACCUGGUA	CGUC GGCU GGCAUG
3725	AUAGAG AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC GGAC CUCUAU

3752	AAUGAC AGAA GCAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUGC UGAC GUCAUU
3771	CACCGC AGAA GCGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCGC CGAC GCGGUG
3783	UCCCCC AGAA GUCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGAC GGUC GGGGGA
3799	CUGGGG AGAA GUAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUAC UGUC CCCCAG
3807	AGACGG AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC AGAC CCGUCU
3812	AUAGGA AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC CGUC UCCUAU
3847	GGGCAG AGAA GUGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCAC UGCU CUGCCC
3852	CCGAAG AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC UGCC CUUCGG
3887	GCACAC AGAA GCCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGGC UGCU GUGUGC
3932	AGACUC AGAA GGUA ACCAGAGAAACA X GUACAUUACCUGGUA	UACC CGUU GAGUCU
3958	ACCGGG AGAA GCAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUGC GGUC CCCGGU
3965	CGUGAA AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC GGUC UUCACG
3992	CGGUAC AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC GGCC GUACCG
4064	GUACGC AGAA GGCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGCC GGCU GCGUAC
4076	CCCUUG AGAA GCGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACGC AGCC CAAGGG
4112	GGCGGC AGAA GAUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAUC UGUU GCCGCC
4163	GUUGGG AGAA GUAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUAC CGAC CCCAAC
4244	UCCACC AGAA GCAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUGC CGAC GGUGGA
4304	AGUCGA AGAA GUUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAAC UGAC UCGACU
4334	GUCCAG AGAA GUGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCAC AGUC CUGGAC
4355	CGCUCC AGAA GUCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGAC GGCU GGAGCG
4366	ACGACG AGAA GCGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCGC GGCU CGUCGU
4441	GUGUUG AGAA GAGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCUC UGUC CAACAC
4621	CCGCUA AGAA GUAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUAC CGAC UAGCGG
4652	UAGAGC AGAA GUUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAAC AGAC GCUCUA
4724	GAAAUC AGAA GUCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGAC AGUC GAUUUC
4734	GAUCCA AGAA GAAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUUC AGCU UGGAUC
4861	CCCGAG AGAA GUUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAAC GGCC CUCGGG
4886	ACACAG AGAA GAAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUUC GGUC CUGUGU
4937	AGUCUC AGAA GGCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGCC CGCU GAGACU
4988	CUGGCA AGAA GGCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGCC CGUC UGCCAG
5059	GUUUGG AGAA GGAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUCC UGUC CCAAAC
5179	GGUUUA AGAA GUAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUAC GGCU UAAACC
5212	CUAUAC AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC UGCU GUAUAG
5231	AUUUUG AGAA GCUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAGC CGUU CAAAAU
5291	CAGGUC AGAA GACA ACCAGAGAAACA X GUACAUUACCUGGUA	UGUC GGCC GACCUG
5294	CUCCAG AGAA GCCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGGC CGAC CUGGAG
5345	GGCCAG AGAA GCAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUGC AGCU CUGGCC
5417	AACAAC AGAA GGCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGCC GGCU GUUGUU
5420	GGGAAC AGAA GCCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGGC UGUU GUUCCC
5509	UCGGCG AGAA GCAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUGC AGCU CGCCGA
5521	UGCUUG AGAA GCUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAGC AGUU CAAGCA
5576	GGGAGC AGAA GCCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGGC CGCU GCUCCC
5579	CACGGG AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC UGCU CCCGUG
5683	UUCCCA AGAA GAGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACUC UGCC UGGGAA
5710	AAUGCC AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC UGAU GGCAUU

5723	GAUAGA AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC AGCC UCUAUC
5736	UGAGCG AGAA GGUG ACCAGAGAAACA X GUACAUUACCUGGUA	CACC AGUC CGCUCA
5740	GUGGUG AGAA GACU ACCAGAGAAACA X GUACAUUACCUGGUA	AGUC CGCU CACCAC
5764	AUGUUG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC UGUU CAACAU
5792	GAGUUG AGAA GCCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGGC UGCU CAACUC
5816	GGCCGA AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC UGCU UCGGCC
5822	CACGAA AGAA GAAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUUC GGCC UUCGUG
5966	GUCCUC AGAA GAGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCUC CGCC GAGGAC
6094	GCUAUC AGAA GGUU ACCAGAGAAACA X GUACAUUACCUGGUA	AACC GGCU GAUAGC
6178	GAGAGG AGAA GAGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACUC AGAU CCUCUC
6189	UGGUGA AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC AGCC UCACCA
6205	UUCAGC AGAA GAGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACUC AGCU GCUGAA
6208	CUCUUC AGAA GCUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAGC UGCU GAAGAG
6243	GCGUGG AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC UGCU CCACGC
6261	GCCACG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC GGCU CGUGGC
6308	CUUGAA AGAA GUCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGAC UGAC UUCAAG
6328	AGCUUG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC AGUC CAAGCU
6340	AAUUUC AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC UGCC GAAAUU
6426	CACAUG AGAA GGUG ACCAGAGAAACA X GUACAUUACCUGGUA	CACC UGCC CAUGUG
6465	UCAUGG AGAA GUUU ACCAGAGAAACA X GUACAUUACCUGGUA	AAAC GGUU CCAUGA
6599	CUCUUC AGAA GCCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGGC UGCU GAAGAG
6692	UUCGGG AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC GGCC CCCGAA
6727	CUGUGC AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC GGUU GCACAG
6753	GGAGAG AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC AGAC CUCUCC
6817	CAUGGG AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC AGCU CCCAUG
6839	UGCCAC AGAA GGUU ACCAGAGAAACA X GUACAUUACCUGGUA	AACC GGAU GUGGCA
6869	GGAGGG AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC CGAC CCCUCC
6939	CUGAAG AGAA GGCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGCC AGCU CUUCAG
7007	GUCAGC AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC GGAC GCUGAC
7013	GAUGAG AGAA GCGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACGC UGAC CUCAUC
7114	GCUCGA AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC CGCU UCGAGC
7148	UGCUGC AGAA GAUA ACCAGAGAAACA X GUACAUUACCUGGUA	UAUC CGUU GCAGCA
7214	GUUGUA AGAA GGGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCCC GGAU UACAAC
7253	GACGUA AGAA GGAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUCC GGAC UACGUC
7291	GUGGUA AGAA GCAA ACCAGAGAAACA X GUACAUUACCUGGUA	UUGC CGCC UACCAC
7315	CGUGGA AGAA GUAU ACCAGAGAAACA X GUACAUUACCUGGUA	AUAC CGCC UCCACG
7337	CAGAAC AGAA GUCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGAC GGUU GUUCUG
7367	CGCCAA AGAA GAAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUUC UGCC UUGGCG
7401	AUCCGG AGAA GCCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGGC AGCU CCGGAU
7407	CCGACG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC GGAU CGUCGG
7415	GUCAAC AGAA GACG ACCAGAGAAACA X GUACAUUACCUGGUA	CGUC GGCC GUUGAC
7418	GCUGUC AGAA GCCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGGC CGUU GACAGC
7439	GGGAGG AGAA GUCG ACCAGAGAAACA X GUACAUUACCUGGUA	CGAC CGCC CCUCCC
7448	GGUCUG AGAA GGAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUCC CGAU CAGACC
7453	UCAGAG AGAA GAUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAUC AGAC CUCUGA
7460	ACCGUC AGAA GAGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCUC UGAC GACGGU

7481	CUCAAC AGAA GAUU ACCAGAGAAACA X GUACAUUACCUGGUA	AAUC UGAC GUUGAG
7535	GCUGAG AGAA GGGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACCC UGAU CUCAGC
7593	UUGAGC AGAA GACG ACCAGAGAAACA X GUACAUUACCUGGUA	CGUC UGCU GCUCAA
7596	ACAUUG AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC UGCU CAAUGU
7627	GGCGUG AGAA GGGC ACCAGAGAAACA X GUACAUUACCUGGUA	GCCC UGAU CACGCC
7660	UUGAUG AGAA GCUU ACCAGAGAAACA X GUACAUUACCUGGUA	AAGC UGCC CAUCAA
7687	UGACGC AGAA GAGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCUC UGCU GCGUCA
7764	CUUGCA AGAA GUCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGAC AGAC UGCAAG
7870	GGGGGC AGAA GCUU ACCAGAGAAACA X GUACAUUACCUGGUA	AAGC UGAC GCCCCC
7956	ACACGG AGAA GAUG ACCAGAGAAACA X GUACAUUACCUGGUA	CAUC CGCU CCGUGU
7975	UCUUCC AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC UGCU GGAAGA
8066	AAGGCG AGAA GGCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGCC AGCU CGCCUU
8087	UCCCAG AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC AGAC CUGGGA
8172	ACUGGA AGAA GUAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUAC GGAU UCCAGU
8262	CAAAGC AGAA GGUG ACCAGAGAAACA X GUACAUUACCUGGUA	CACC CGCU GCUUUG
8265	AGUCAA AGAA GCGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCGC UGCU UUGACU
8374	AUGUAG AGAA GCUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAGC GGCU CUACAU
8395	GAAUUA AGAA GGGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCCC UGAC UAAUUC
8452	CUAGUC AGAA GCAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUGC UGAC GACUAG
8501	UCGACA AGAA GCAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUGC GGCC UGUCGA
8505	CAGCUC AGAA GGCC ACCAGAGAAACA X GUACAUUACCUGGUA	GGCC UGUC GAGCUG
8639	GGGGGG AGAA GAGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACUC UGCC CCCCCC
8656	GGUUGG AGAA GGUC ACCAGAGAAACA X GUACAUUACCUGGUA	GACC CGCC CCAACC
8711	GUGCGC AGAA GACA ACCAGAGAAACA X GUACAUUACCUGGUA	UGUC GGUC GCGCAC
8911	UUUUCA AGAA GUUC ACCAGAGAAACA X GUACAUUACCUGGUA	GAAC AGCU UGAAAA
8935	CCGUAG AGAA GACA ACCAGAGAAACA X GUACAUUACCUGGUA	UGUC AGAU CUACGG
8980	UGAAUG AGAA GAGG ACCAGAGAAACA X GUACAUUACCUGGUA	CCUC AGAU CAUUCA
9082	CGCAAG AGAA GUAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUAC CGCC CUUGCG
9133	CCUUGG AGAA GUAG ACCAGAGAAACA X GUACAUUACCUGGUA	CUAC UGUC CCAAGG
9218	GGACGC AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC GGCC GCGUCC
9229	AAGUCC AGAA GGGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCCC AGCU GGACUU
9243	CGAACC AGAA GGAC ACCAGAGAAACA X GUACAUUACCUGGUA	GUCC AGCU GGUUCG
9285	GAGACA AGAA GUGA ACCAGAGAAACA X GUACAUUACCUGGUA	UCAC AGCC UGUCUC
9289	GCACGA AGAA GGCU ACCAGAGAAACA X GUACAUUACCUGGUA	AGCC UGUC UCGUGC
9300	AGCGGG AGAA GGCA ACCAGAGAAACA X GUACAUUACCUGGUA	UGCC CGAC CCCGCU
9306	UAAACC AGAA GGGU ACCAGAGAAACA X GUACAUUACCUGGUA	ACCC CGCU GGUUUA
9358	UUGGGG AGAA GGUA ACCAGAGAAACA X GUACAUUACCUGGUA	UACC UGCU CCCCAA

Where "X" represents stem IV region of a HP ribozyme (Berzal-Herranz *et al.*, 1993, *EMBO.J.* 12, 2567). The length of stem IV may be 2 base-pairs.

Table VIII: Additional HCV Conserved Hammerhead ribozyme and target sequence

Nos.	Name*	Pos.†	Ribozyme	Substrate
1	HCV.C-48	278	UUGGUGU CUGAUGAG X CGAA ACGUUUG	CAAACGU A ACACCAA
2	HCV.C-60	290	UGUGGGC CUGAUGAG X CGAA ACGGUUG	CAACCGU C GCCCACA
3	HCV.C-175	405	AGGUUGU CUGAUGAG X CGAA ACCGCUC	GAGCGGU C ACAACCU
4	HCV.3-118	9418	AAAAAAA CUGAUGAG X CGAA AAAAAAA	UUUUUUU U UUUUUUU
5	HCV.3-145	9445	UAAGAUG CUGAUGAG X CGAA AGCCACC	GGUGGCU C CAUCUUA
6	HCV.3-149	9449	GGGCUAA CUGAUGAG X CGAA AUGGAGC	GCUCCAU C UUAGCCC
7	HCV.3-151	9451	UAGGGCU CUGAUGAG X CGAA AGAUGGA	UCCAUCU U AGCCCUA
8	HCV.3-152	9452	CUAGGGC CUGAUGAG X CGAA AAGAUGG	CCAUCUU A GCCCUAG
9	HCV.3-158	9458	CCGUGAC CUGAUGAG X CGAA AGGGCUA	UAGCCCU A GUCACGG
10	HCV.3-161	9461	UAGCCGU CUGAUGAG X CGAA ACUAGGG	CCCUAGU C ACGGCUA
11	HCV.3-168	9468	UCACAGC CUGAUGAG X CGAA AGCCGUG	CACGGCU A GCUGUGA
12	HCV.3-181	9481	GCUCACG CUGAUGAG X CGAA ACCUUUC	GAAAGGU C CGUGAGC

Where "X" represents stem II region of a HH ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20: 3252). The length of stem II may be 2 base-pairs.

Core Reference Sequence for Nos. 1-3 = HPCCOPR (Acc#L38318) 1-600 bp
*-Nucleotide 231 (8 nucleotide upstream of the initiator ATG) has been designated as "1" for the purpose of numbering ribozyme sites in the core protein coding region.

3'-NCR Reference Sequence for Nos. 4-12= D85516 (Acc#D85516) 9301-9535 bp
*- Nucleotide 9301 has been designated as "1" for the purpose of numbering ribozyme sites in the 3'NCR.

†- position number reflects the reference sequence from HPCCOPR.

Table IX. Inhibition of HCV RNA in OST7 cells Using Multiple Ribozyme Motifs

Motif	RPI Number	F_{luc}/R_{luc}	SEM	Sequence
RPI Motif I	Irrelevant Control	0.22	0.03	auccuUGAU$_s$GGCAUACACUAUGCGCGaugaucugcaB
RPI Motif I	18738	0.13	0.03	acacuuGAU$_s$ggcauGcacuaugcgcgauacuaacgcB
RPI Motif I	18739	0.15	0.01	cacgauGAU$_s$ggcauGcacuaugcgcgacucauacuaB
RPI Motif I	18740	0.15	0.01	ggcuguGAU$_s$ggcauGcacuaugcgcgacgacacucaB
RPI Motif I	18746	0.10	0.02	cccaauGAU$_s$ggcauGcacuaugcgcgacuacucggcB
RPI Motif I	18747	0.16	0.02	uuucguGAU$_s$ggcauGcacuaugcgcggacccaacacB
RPI Motif I	18750	0.15	0.03	ucagguGAU$_s$ggcauGcacuaugcgcgaguaccacaaB
RPI Motif I	18754	0.12	0.01	gcacuuGAU$_s$ggcauGcacuaugcgcggcaagcacccB
RPI Motif II	SAC	1.10	0.32	a$_s$u$_s$u$_s$c$_s$ca cUAGuGaggcguuagccGau Acgcga B
RPI Motif II	20339	0.85	0.01	u$_s$c$_s$c$_s$u$_s$caccUGAuGaggccguuaggccGaaIgggaguB
RPI Motif II	20350	1.04	0.05	g$_s$u$_s$c$_s$c$_s$uggcUGAuGaggccguuaggccGaaIgcugcaB
RPI Motif III	Irrelevant Control	1.28	0.07	ggaaaggugugcaaCCGgaggaaacucCCUUCAAGGACAUCGUCCGGGacggcB
RPI Motif III	18704	0.37	0.07	uuccgcagaCGgaggaaacucCCUUCAAGGACGAAAGUCCGGGacuauggB
RPI Motif III	18705	0.42	0.10	ccgcagaCGgaggaaacucCCUUCAAGGACGAAAGUCCGGGacuauggB
RPI Motif III	18700	0.61	0.16	cagguaguaCGgaggaaacucCCUUCAAGGACAUCGUCCGGGacaaggB
RPI Motif III	18701	0.54	0.10	gcacggucUaGgaggaaacucCCUUCAAGGACAUCGUCCGGGgagaccB
RPI Motif III	18835	0.54	0.04	guguacucacGgaggaaacucCCUUCAAGGACAUCGUCCGGGgguucB

Chemical Modifications are indicated as follows:
Lower case = 2'-O-Methyl
Bold (non-italicized): 2'-NH_2
U = 2'-C-Allyl-U
G,A= ribo G,A
s = phosphorothioate linkages
B = inverted abasic
I = ribo Inosine

Table X: Anti HCV minus strand Stabilized Ribozyme and Target Sequence

RPI No.	Ribozyme Alias	Ribozyme Sequence	Target Sequence
14961	HCV.5nc-34 Rz-7 allyl stab1	$g_sg_su_sc_s$ucg cUGAuGaggccguuaggccGaa Agaccgu B	ACGGUCU A CGAGACC
14962	HCV.5nc-43 Rz-7 allyl stab1	$g_sc_sc_sc_s$cgg cUGAuGaggccguuaggccGaa Aggucuc B	GAGACCU C CCGGGGC
14963	HCV.5nc-54 Rz-7 allyl stab1	$u_sg_sc_su_s$ugc cUGAuGaggccguuaggccGaa Agugccc B	GGGCACU C GCAAGCA
14964	HCV.5nc-66 Rz-7 allyl stab1	$u_sg_sc_sc_s$uga cUGAuGaggccguuaggccGaa Agggugc B	GCACCCU A UCAGGCA
14965	HCV.5nc-88 Rz-7 allyl stab1	$g_su_sc_sg_s$cga cUGAuGaggccguuaggccGaa Aggccuu B	AAGGCCU U UCGCGAC
14966	HCV.5nc-88b Rz-7 allyl stab1	$g_su_su_sg_s$cga cUGAuGaggccguuaggccGaa Aggccuu B	AAGGCCU U UCGCAAC
14967	HCV.5nc-107 Rz-7 allyl stab1	$g_sc_su_sa_s$gcc cUGAuGaggccguuaggccGaa Aguagug B	CACUACU C GGCUAGC
14968	HCV.5nc-162 Rz-7 allyl stab1	$u_su_su_sc_s$uug cUGAuGaggccguuaggccGaa Aucaacc B	GGUUGAU C CAAGAAA
14969	HCV.5nc-162b Rz-7 allyl stab1	$u_su_su_sc_s$uug cUGAuGaggccguuaggccGaa Auaaacc B	GGUUUAU C CAAGAAA
14970	HCV.5nc-192 Rz-7 allyl stab1	$u_sa_sc_sa_s$ccg cUGAuGaggccguuaggccGaa Aauugcc B	GGCAAUU C CGGUGUA
14971	HCV.5nc-199 Rz-7 allyl stab1	$c_sg_sg_su_s$gag cUGAuGaggccguuaggccGaa Acaccgg B	CCGGUGU A CUCACCG
14972	HCV.5nc-202 Rz-7 allyl stab1	$a_sa_sc_sc_s$ggu cUGAuGaggccguuaggccGaa Aguacac B	GUGUACU C ACCGGUU
14973	HCV.5nc-222 Rz-7 allyl stab1	$a_sg_sa_sg_s$cca cUGAuGaggccguuaggccGaa Agugguc B	GACCACU A UGGCUCU
14974	HCV.5nc-265 Rz-7 allyl stab1	$u_su_sa_sg_s$uau cUGAuGaggccguuaggccGaa Agugucg B	CGACACU C AUACUAA
14975	HCV.5nc-33 CHz-7 allyl stab1	$g_su_sc_su_s$cgu cUGAuGaggccguuaggccGaa Iaccgug B	CACGGUC U ACGAGAC
14976	HCV.5nc-41 CHz-7 allyl stab1	$c_sc_sc_sg_s$gga cUGAuGaggccguuaggccGaa Iucucgu B	ACGAGAC C UCCCGGG
14977	HCV.5nc-42 CHz-7 allyl stab1	$c_sc_sc_sc_s$ggg cUGAuGaggccguuaggccGaa Igucucg B	CGAGACC U CCCGGGG
14978	HCV.5nc-44 CHz-7 allyl stab1	$u_sg_sc_sc_s$ccg cUGAuGaggccguuaggccGaa Iaggucu B	AGACCUC C CGGGGCA
14979	HCV.5nc-45 CHz-7 allyl stab1	$g_su_sg_sc_s$ccc cUGAuGaggccguuaggccGaa Igagguc B	GACCUCC C GGGGCAC
14980	HCV.5nc-51 CHz-7 allyl stab1	$u_su_sg_sc_s$gag cUGAuGaggccguuaggccGaa Iccccgg B	CCGGGGC A CUCGCAA
14981	HCV.5nc-53 CHz-7 allyl stab1	$g_sc_su_su_s$gcg cUGAuGaggccguuaggccGaa Iugcccc B	GGGGCAC U CGCAAGC
14982	HCV.5nc-57 CHz-7 allyl stab1	$g_sg_sg_su_s$gcu cUGAuGaggccguuaggccGaa Icgagug B	CACUCGC A AGCACCC
14983	HCV.5nc-61 CHz-7 allyl stab1	$g_sa_su_sa_s$ggg cUGAuGaggccguuaggccGaa Icuugcg B	CGCAAGC A CCCUAUC
14984	HCV.5nc-63 CHz-7 allyl stab1	$c_su_sg_sa_s$uag cUGAuGaggccguuaggccGaa Iugcuug B	CAAGCAC C CUAUCAG
14985	HCV.5nc-64 CHz-7 allyl stab1	$c_sc_su_sg_s$aua cUGAuGaggccguuaggccGaa Igugcuu B	AAGCACC C UAUCAGG
14986	HCV.5nc-65 CHz-7 allyl stab1	$g_sc_sc_su_s$gau cUGAuGaggccguuaggccGaa Iggugcu B	AGCACCC U AUCAGGC
14987	HCV.5nc-73 CHz-7 allyl stab1	$g_su_sg_sg_s$uac cUGAuGaggccguuaggccGaa Iccugau B	AUCAGGC A GUACCAC
14988	HCV.5nc-78 CHz-7 allyl stab1	$g_sc_sc_su_s$ugu cUGAuGaggccguuaggccGaa Iuacugc B	GCAGUAC C ACAAGGC

14989	HCV.5nc-79 CHz-7 allyl stab1	$g_sg_sc_sc_s$uug cUGAuGaggccguuaggccGaa Iguacug B	CAGUACC A CAAGGCC
14990	HCV.5nc-81 CHz-7 allyl stab1	$a_sa_sg_sg_s$ccu cUGAuGaggccguuaggccGaa Iugguac B	GUACCAC A AGGCCUU
14991	HCV.5nc-87 CHz-7 allyl stab1	$u_sc_sg_sc_s$gaa cUGAuGaggccguuaggccGaa Igccuug B	CAAGGCC U UUCGCGA
14992	HCV.5nc-87b CHz-7 allyl stab1	$u_su_sg_sc_s$gaa cUGAuGaggccguuaggccGaa Igccuug B	CAAGGCC U UUCGCAA
14993	HCV.5nc-101 CHz-7 allyl stab1	$c_sg_sa_sg_s$uag cUGAuGaggccguuaggccGaa Iuugggu B	ACCCAAC A CUACUCG
14994	HCV.5nc-103 CHz-7 allyl stab1	$g_sc_sc_sg_s$agu cUGAuGaggccguuaggccGaa Iuguugg B	CCAACAC U ACUCGGC
14995	HCV.5nc-106 CHz-7 allyl stab1	$c_su_sa_sg_s$ccg cUGAuGaggccguuaggccGaa Iuagugu B	ACACUAC U CGGCUAG
14996	HCV.5nc-111 CHz-7 allyl stab1	$g_sa_sc_su_s$gcu cUGAuGaggccguuaggccGaa Iccgagu B	ACUCGGC U AGCAGUC
14997	HCV.5nc-119 CHz-7 allyl stab1	$c_sc_sc_sc_s$gcg cUGAuGaggccguuaggccGaa Iacugcu B	AGCAGUC U CGCGGGG
14998	HCV.5nc-129 CHz-7 allyl stab1	$u_su_sg_sg_s$gcg cUGAuGaggccguuaggccGaa Icccccg B	CGGGGGC A CGCCCAA
14999	HCV.5nc-163 CHz-7 allyl stab1	$c_su_su_su_s$cuu cUGAuGaggccguuaggccGaa Iaucaac B	GUUGAUC C AAGAAAG
15000	HCV.5nc-163b CHz-7 allyl stab1	$c_su_su_su_s$cuu cUGAuGaggccguuaggccGaa Iauaaac B	GUUUAUC C AAGAAAG
15001	HCV.5nc-164 CHz-7 allyl stab1	$c_sc_su_su_s$ucu cUGAuGaggccguuaggccGaa Igaucaa B	UUGAUCC A AGAAAGG
15002	HCV.5nc-164b CHz-7 allyl stab1	$c_sc_su_su_s$ucu cUGAuGaggccguuaggccGaa Igauaaa B	UUUAUCC A AGAAAGG
15003	HCV.5nc-193 CHz-7 allyl stab1	$g_su_sa_sc_s$acc cUGAuGaggccguuaggccGaa Iaauugc B	GCAAUUC C GGUGUAC
15004	HCV.5nc-201 CHz-7 allyl stab1	$a_sc_sc_sg_s$gug cUGAuGaggccguuaggccGaa Iuacacc B	GGUGUAC U CACCGGU
15005	HCV.5nc-203 CHz-7 allyl stab1	$g_sa_sa_sc_s$cgg cUGAuGaggccguuaggccGaa Iaguaca B	UGUACUC A CCGGUUC
15006	HCV.5nc-205 CHz-7 allyl stab1	$c_sg_sg_sa_s$acc cUGAuGaggccguuaggccGaa Iugagua B	UACUCAC C GGUUCCG
15007	HCV.5nc-211 CHz-7 allyl stab1	$g_sg_su_sc_s$ugc cUGAuGaggccguuaggccGaa Iaaccgg B	CCGGUUC C GCAGACC
15008	HCV.5nc-214 CHz-7 allyl stab1	$a_sg_su_sg_s$guc cUGAuGaggccguuaggccGaa Icggaac B	GUUCCGC A GACCACU
15009	HCV.5nc-218 CHz-7 allyl stab1	$c_sc_sa_su_s$agu cUGAuGaggccguuaggccGaa Iucugcg B	CGCAGAC C ACUAUGG
15010	HCV.5nc-219 CHz-7 allyl stab1	$g_sc_sc_sa_s$uag cUGAuGaggccguuaggccGaa Igucugc B	GCAGACC A CUAUGGC
15011	HCV.5nc-221 CHz-7 allyl stab1	$g_sa_sg_sc_s$cau cUGAuGaggccguuaggccGaa Iuggucu B	AGACCAC U AUGGCUC
15012	HCV.5nc-227 CHz-7 allyl stab1	$c_sc_sg_sg_s$gag cUGAuGaggccguuaggccGaa Iccauag B	CUAUGGC U CUCCCGG
15013	HCV.5nc-229 CHz-7 allyl stab1	$u_sc_sc_sc_s$ggg cUGAuGaggccguuaggccGaa Iagccau B	AUGGCUC U CCCGGGA
15014	HCV.5nc-231 CHz-7 allyl stab1	$c_sc_su_sc_s$ccg cUGAuGaggccguuaggccGaa Iagagcc B	GGCUCUC C CGGGAGG
15015	HCV.5nc-232 CHz-7 allyl stab1	$c_sc_sc_su_s$ccc cUGAuGaggccguuaggccGaa Igagagc B	GCUCUCC C GGGAGGG
15016	HCV.5nc-266 CHz-7 allyl stab1	$g_su_su_sa_s$gua cUGAuGaggccguuaggccGaa Iaguguc B	GACACUC A UACUAAC
15017	HCV.5nc-270 CHz-7 allyl stab1	$u_sg_sg_sc_s$guu cUGAuGaggccguuaggccGaa Iuaugag B	CUCAUAC U AACGCCA
15018	HCV.5-31 CHz-7 allyl stab1	$u_sc_sa_sc_s$agg cUGAuGaggccguuaggccGaa Iagugau B	AUCACUC C CCUGUGA
15019	HCV.5-32 CHz-7 allyl stab1	$c_su_sc_sa_s$cag cUGAuGaggccguuaggccGaa Igaguga B	UCACUCC C CUGUGAG
15020	HCV.5-33 CHz-7 allyl stab1	$c_sc_su_sc_s$aca cUGAuGaggccguuaggccGaa Iggagug B	CACUCCC C UGUGAGG

15021	HCV.5-34 CHz-7 allyl stab1	$u_s c_s c_s u_s$cac cUGAuGaggccguuaggccGaa Igggagu B	ACUCCCC U GUGAGGA
15022	HCV.5-44 CHz-7 allyl stab1	$a_s g_s a_s c_s$agu cUGAuGaggccguuaggccGaa Iuuccuc B	GAGGAAC U ACUGUCU
15023	HCV.5-47 CHz-7 allyl stab1	$u_s g_s a_s a_s$gac cUGAuGaggccguuaggccGaa Iuaguuc B	GAACUAC U GUCUUCA
15024	HCV.5-51 CHz-7 allyl stab1	$u_s g_s c_s g_s$uga cUGAuGaggccguuaggccGaa Iacagua B	UACUGUC U UCACGCA
15025	HCV.5-54 CHz-7 allyl stab1	$u_s u_s c_s u_s$gcg cUGAuGaggccguuaggccGaa Iaagaca B	UGUCUUC A CGCAGAA
15026	HCV.5-58 CHz-7 allyl stab1	$c_s g_s c_s u_s$uuc cUGAuGaggccguuaggccGaa Icgugaa B	UUCACGC A GAAAGCG
15027	HCV.5-68 CHz-7 allyl stab1	$c_s a_s u_s g_s$gcu cUGAuGaggccguuaggccGaa Iacgcuu B	AAGCGUC U AGCCAUG
15028	HCV.5-72 CHz-7 allyl stab1	$a_s c_s g_s c_s$cau cUGAuGaggccguuaggccGaa Icuagac B	GUCUAGC C AUGGCGU
15029	HCV.5-73 CHz-7 allyl stab1	$a_s a_s c_s g_s$cca cUGAuGaggccguuaggccGaa Igcuaga B	UCUAGCC A UGGCGUU
15030	HCV.5-97 CHz-7 allyl stab1	$u_s g_s g_s a_s$ggc cUGAuGaggccguuaggccGaa Icacgac B	GUCGUGC A GCCUCCA
15031	HCV.5-100 CHz-7 allyl stab1	$u_s c_s c_s u_s$gga cUGAuGaggccguuaggccGaa Icugcac B	GUGCAGC C UCCAGGA
15032	HCV.5-101 CHz-7 allyl stab1	$g_s u_s c_s c_s$ugg cUGAuGaggccguuaggccGaa Igcugca B	UGCAGCC U CCAGGAC
15033	HCV.5-103 CHz-7 allyl stab1	$g_s g_s g_s u_s$ccu cUGAuGaggccguuaggccGaa Iaggcug B	CAGCCUC C AGGACCC
15034	HCV.5-104 CHz-7 allyl stab1	$g_s g_s g_s g_s$ucc cUGAuGaggccguuaggccGaa Igaggcu B	AGCCUCC A GGACCCC
15035	HCV.5-109 CHz-7 allyl stab1	$g_s a_s g_s g_s$ggg cUGAuGaggccguuaggccGaa Iuccugg B	CCAGGAC C CCCCCUC
15036	HCV.5-110 CHz-7 allyl stab1	$g_s g_s a_s g_s$ggg cUGAuGaggccguuaggccGaa Iguccug B	CAGGACC C CCCCUCC
15037	HCV.5-111 CHz-7 allyl stab1	$g_s g_s g_s a_s$ggg cUGAuGaggccguuaggccGaa Igguccu B	AGGACCC C CCCUCCC
15038	HCV.5-112 CHz-7 allyl stab1	$c_s g_s g_s g_s$agg cUGAuGaggccguuaggccGaa Igggucc B	GGACCCC C CCUCCCG
15039	HCV.5-113 CHz-7 allyl stab1	$c_s c_s g_s g_s$gag cUGAuGaggccguuaggccGaa Igggguc B	GACCCCC C CUCCCGG
15040	HCV.5-114 CHz-7 allyl stab1	$c_s c_s c_s g_s$gga cUGAuGaggccguuaggccGaa Igggggu B	ACCCCCC C UCCCGGG
15041	HCV.5-115 CHz-7 allyl stab1	$u_s c_s c_s c_s$ggg cUGAuGaggccguuaggccGaa Igggggg B	CCCCCCC U CCCGGGA
15042	HCV.5-117 CHz-7 allyl stab1	$u_s c_s u_s c_s$ccg cUGAuGaggccguuaggccGaa Iaggggg B	CCCCCUC C CGGGAGA
15043	HCV.5-118 CHz-7 allyl stab1	$c_s u_s c_s u_s$ccc cUGAuGaggccguuaggccGaa Igagggg B	CCCCUCC C GGGAGAG
15044	HCV.5-127 CHz-7 allyl stab1	$c_s c_s a_s c_s$uau cUGAuGaggccguuaggccGaa Icucucc B	GGAGAGC C AUAGUGG
15045	HCV.5-128 CHz-7 allyl stab1	$a_s c_s c_s a_s$cua cUGAuGaggccguuaggccGaa Igcucuc B	GAGAGCC A UAGUGGU
15046	HCV.5-137 CHz-7 allyl stab1	$g_s u_s u_s c_s$cgc cUGAuGaggccguuaggccGaa Iaccacu B	AGUGGUC U GCGGAAC
15047	HCV.5-145 CHz-7 allyl stab1	$a_s c_s u_s c_s$acc cUGAuGaggccguuaggccGaa Iuuccgc B	GCGGAAC C GGUGAGU
15048	HCV.5-155 CHz-7 allyl stab1	$a_s u_s u_s c_s$cgg cUGAuGaggccguuaggccGaa Iuacuca B	UGAGUAC A CCGGAAU
15049	HCV.5-157 CHz-7 allyl stab1	$c_s a_s a_s u_s$ucc cUGAuGaggccguuaggccGaa Iuguacu B	AGUACAC C GGAAUUG
15050	HCV.5-181 CHz-7 allyl stab1	$c_s a_s a_s g_s$aaa cUGAuGaggccguuaggccGaa Iacccgg B	CCGGGUC C UUUCUUG
15051	HCV.5-182 CHz-7 allyl stab1	$c_s c_s a_s a_s$gaa cUGAuGaggccguuaggccGaa Igacccg B	CGGGUCC U UUCUUGG
15052	HCV.5-186 CHz-7 allyl stab1	$u_s g_s a_s u_s$cca cUGAuGaggccguuaggccGaa Iaaagga B	UCCUUUC U UGGAUCA

Table X

15053	HCV.5-268 CHz-7 allyl stab1	$a_s c_s c_s a_s$caa cUGAuGaggccguuaggccGaa Iccuuuc B	GAAAGGC C UUGUGGU
15054	HCV.5-278 CHz-7 allyl stab1	$a_s u_s c_s a_s$ggc cUGAuGaggccguuaggccGaa Iuaccac B	GUGGUAC U GCCUGAU
15055	HCV.5-293 CHz-7 allyl stab1	$a_s c_s u_s c_s$gca cUGAuGaggccguuaggccGaa Icacccu B	AGGGUGC U UGCGAGU
15056	HCV.5-314 CHz-7 allyl stab1	$g_s u_s c_s u_s$acg cUGAuGaggccguuaggccGaa Iaccucc B	GGAGGUC U CGUAGAC

s = phosphorothioate
B = inverted deoxyabasic residue
Lower case = 2'-O-Methyl
UPPER CASE = ribo
I = inosine
U = 2'-deoxy-2'-C-allyl Uridine

CLAIMS

What we claim is:

1. An enzymatic nucleic acid molecule which specifically cleaves minus strand RNA derived from hepatitis C virus (HCV), wherein the binding arms of said enzymatic nucleic acid molecule comprises sequences complementary to any of substrate sequences defined in Table X.

2. An enzymatic nucleic acid molecule which specifically cleaves minus strand RNA derived from hepatitis C virus (HCV), wherein said enzymatic nucleic acid molecule comprises sequences defined as ribozyme sequences in Table X.

3. An enzymatic nucleic acid molecule which selectively cleaves RNA derived from HCV, wherein said enzymatic nucleic acid molecule is selected from the group consisting of inozyme, G-cleaver, DNAzyme, Amberzyme, and Zinzyme motifs.

4. The enzymatic nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule cleaves plus strand RNA derived from HCV.

5. The enzymatic nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule cleaves minus strand RNA derived from HCV.

6. The enzymatic nucleic acid molecule of claim 3, wherein said inozyme enzymatic nucleic acid molecule comprises a stem II region of length greater than or equal to 2 base pairs.

7. The enzymatic nucleic acid molecule of claim 1, wherein said enzymatic nucleic acid molecule is selected from the group consisting of hammerhead (HH), G-cleaver, Inozyme, DNAzyme, Amberzyme, and Zinzyme motifs.

8. The enzymatic nucleic acid molecule of any of claims 1 and 3, wherein said enzymatic nucleic acid comprises between 12 and 100 bases complementary to said RNA derived from HCV.

9. The enzymatic nucleic acid molecule of any of claims 1 and 3, wherein said enzymatic nucleic acid comprises between 14 and 24 bases complementary to said RNA derived from HCV.

10. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of any of claims 1 and 3.

11. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 1 and 3.

12. The mammalian cell of claim 11, wherein said mammalian cell is a human cell.

13. An expression vector comprising nucleic acid sequence encoding at least one enzymatic nucleic acid molecule of claims 1 or 3, in a manner which allows expression of that enzymatic nucleic acid molecule.

14. A mammalian cell including an expression vector of claim 13.

15. The mammalian cell of claim 14, wherein said mammalian cell is a human cell.

16. A method for treatment of cirrhosis, liver failure or hepatocellular carcinoma comprising the step of administering to a patient the enzymatic nucleic acid molecule of any of claims 1 and 3 under conditions suitable for said treatment.

17. A method for treatment of cirrhosis, liver failure and/or hepatocellular carcinoma comprising the step of administering to a patient the expression vector of claim 13 under conditions suitable for said treatment.

18. A method of treatment of a patient having a condition associated with HCV infection, comprising contacting cells of said patient with the nucleic acid molecule of any of claims 1 and 3, and further comprising the use of one or more drug therapies under conditions suitable for said treatment.

19. A method for inhibiting HCV replication in a mammalian cell comprising the step of administering to said cell the enzymatic nucleic acid molecule of any of claims 1 and 3 under conditions suitable for said inhibition.

20. A method of cleaving a separate RNA molecule comprising, contacting the enzymatic nucleic acid molecule of any of claims 1 and 3 with said separate RNA molecule under conditions suitable for the cleavage of said separate RNA molecule.

21. The method of claim 20, wherein said cleavage is carried out in the presence of a divalent cation.

22. The method of claim 21, wherein said divalent cation is Mg^{2+}.

23. The nucleic acid molecule of claims 1 or 3, wherein said nucleic acid is chemically synthesized.

24. The expression vector of claim 13, wherein said vector comprises:

a. a transcription initiation region;

b. a transcription termination region;

c. a nucleic acid sequence encoding at least one said nucleic acid molecule; and

wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

25. The expression vector of claim 13, wherein said vector comprises:

a. a transcription initiation region;

b. a transcription termination region;

c. an open reading frame;

d. a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and

wherein said sequence is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

26. The expression vector of claim 13, wherein said vector comprises:

a. a transcription initiation region;

b. a transcription termination region;

c. an intron;

d. a nucleic acid sequence encoding at least one said nucleic acid molecule; and

wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

27. The expression vector of claim 13, wherein said vector comprises:

a. a transcription initiation region;

b. a transcription termination region;

c. an intron;

d. an open reading frame;

e. a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and

wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

28. The enzymatic nucleic acid molecule of claims 1 or 3, wherein said enzymatic nucleic acid comprises at least one 2'-sugar modification.

29. The enzymatic nucleic acid molecule of claims 1 or 3, wherein said enzymatic nucleic acid comprises at least one nucleic acid base modification.

30. The enzymatic nucleic acid molecule of claims 1 or 3, wherein said enzymatic nucleic acid comprises at least one phosphate modification.

31. The method of claim 18, wherein said drug therapies is type I interferon.

32. The method of claim 31, wherein said type I interferon and the enzymatic nucleic acid molecule are administered simultaneously.

33. The method of claim 31, wherein said type I interferon and enzymatic nucleic acid molecule are administered separately.

34. The method of claim 31, wherein said type I interferon is interferon alpha.

35. The method of claim 31, wherein said type I interferon is interferon beta.

36. The method of claim 31, wherein said type I interferon is interferon gamma.

37. The method of claim 31, wherein said type I interferon is consensus interferon.

Abstract of the Disclosure

Enzymatic nucleic acid molecules which modulate the expression and/or replication of hepatitis C virus (HCV).

Figure 1: Ribozyme Motifs

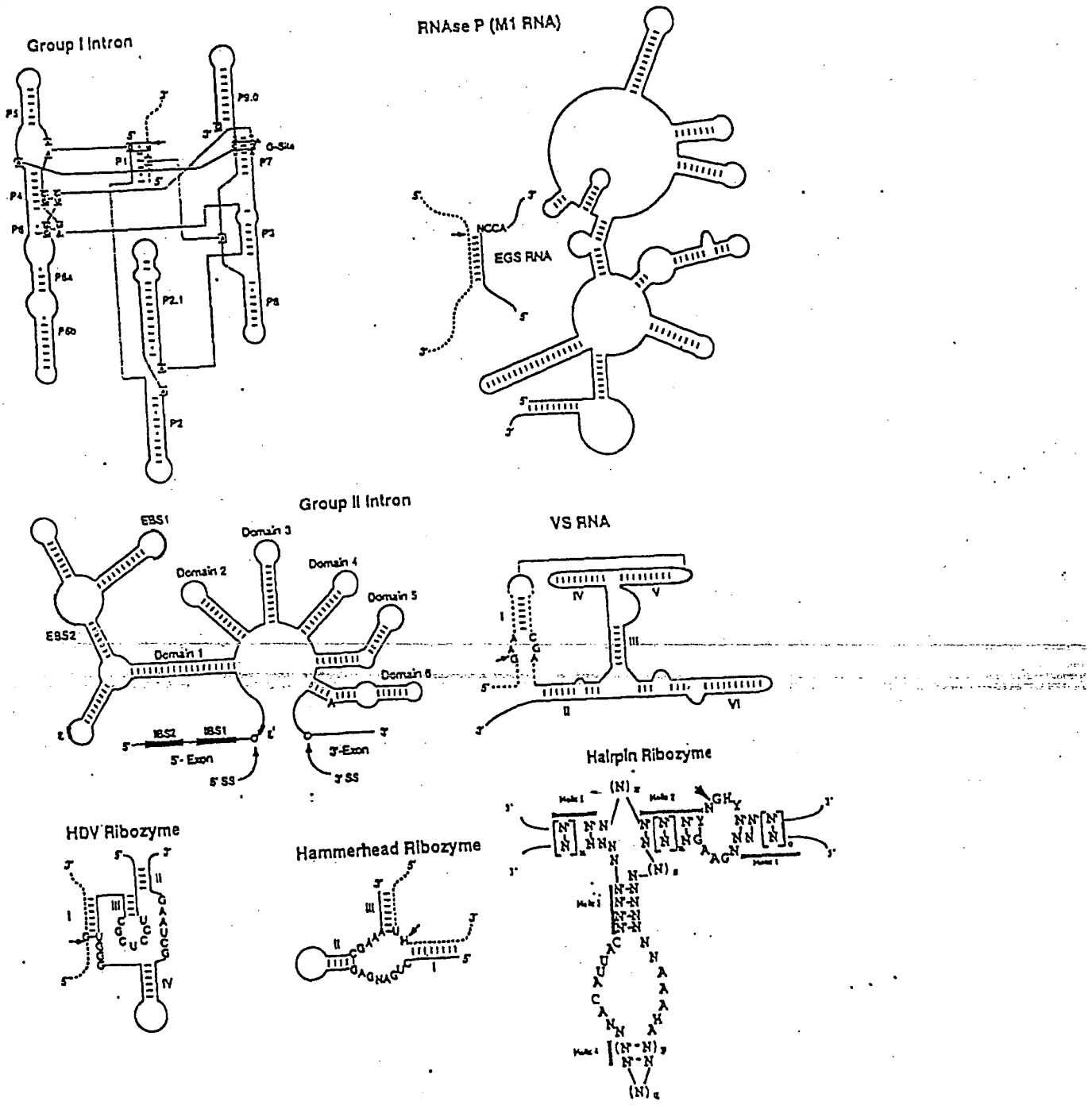


Figure 2. *In vitro* Cleavage of 5'HCV UTR Transcripts From Four HCV Genotypes

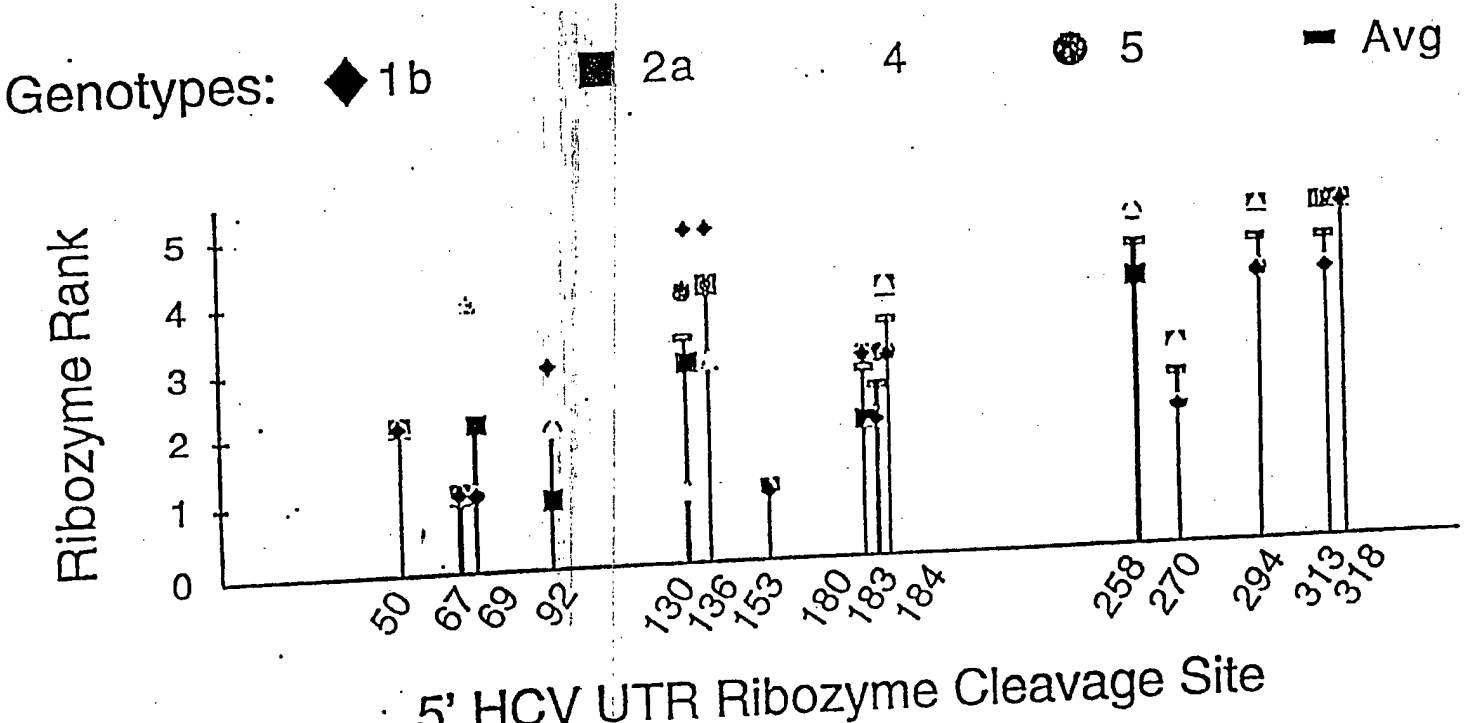


FIGURE 3. Dual Reporter System for Cytoplasmic HCV Target

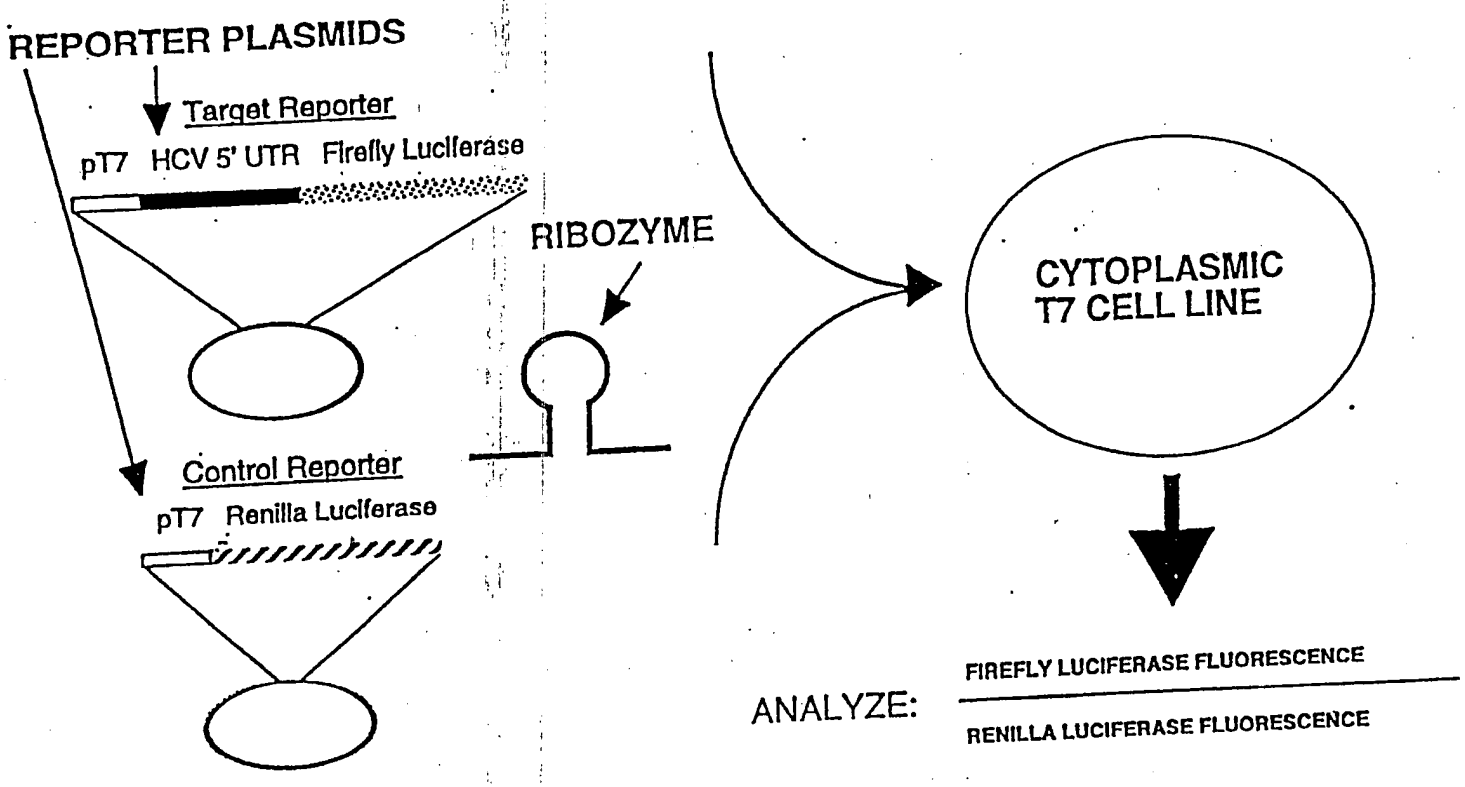


Figure 4. Ribozyme Mediated Reduction of Luciferase Activity in OST-7 Cells

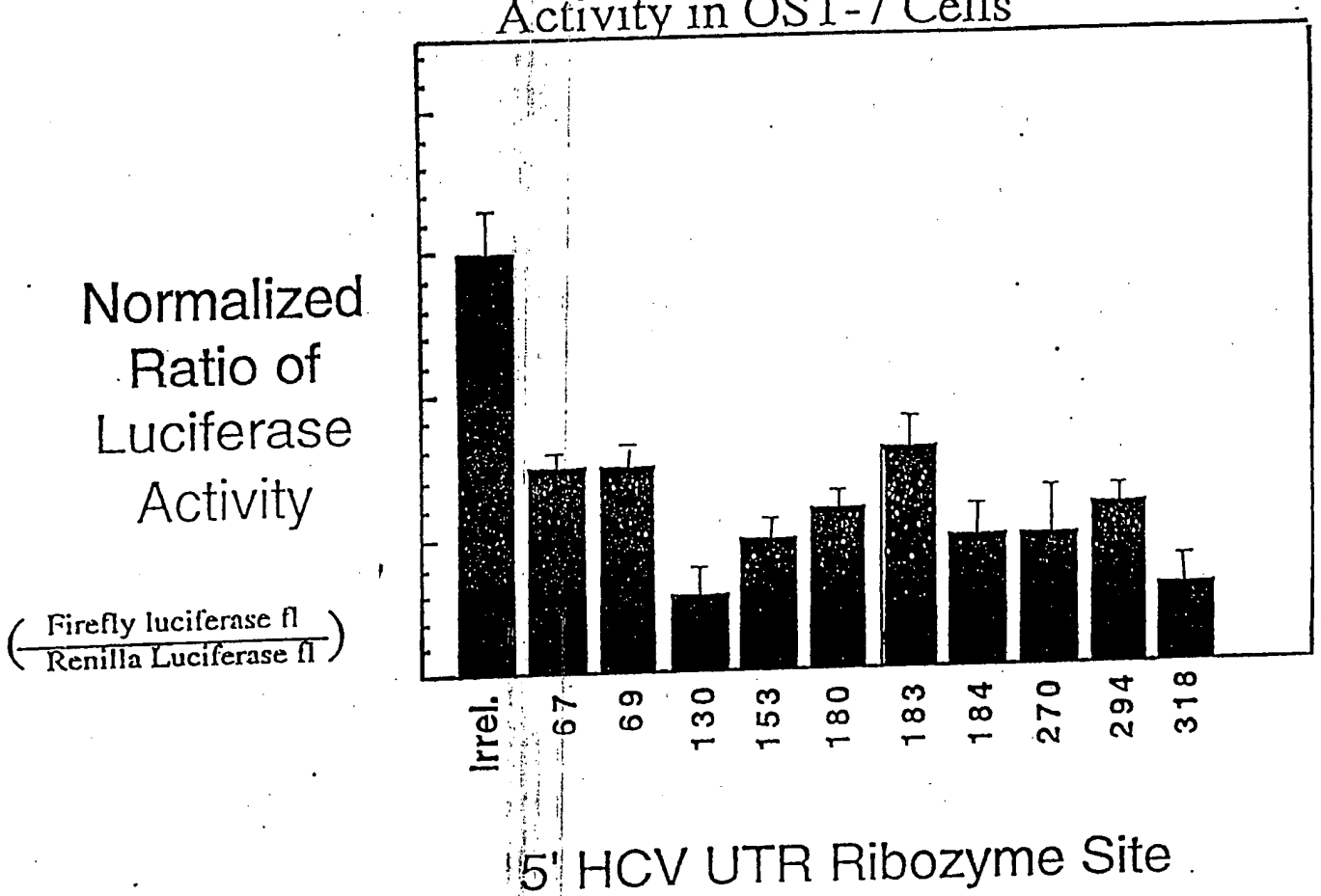


Figure 5.Ribozyme Mediated Reduction of Luciferase Activity In OST-7 cells

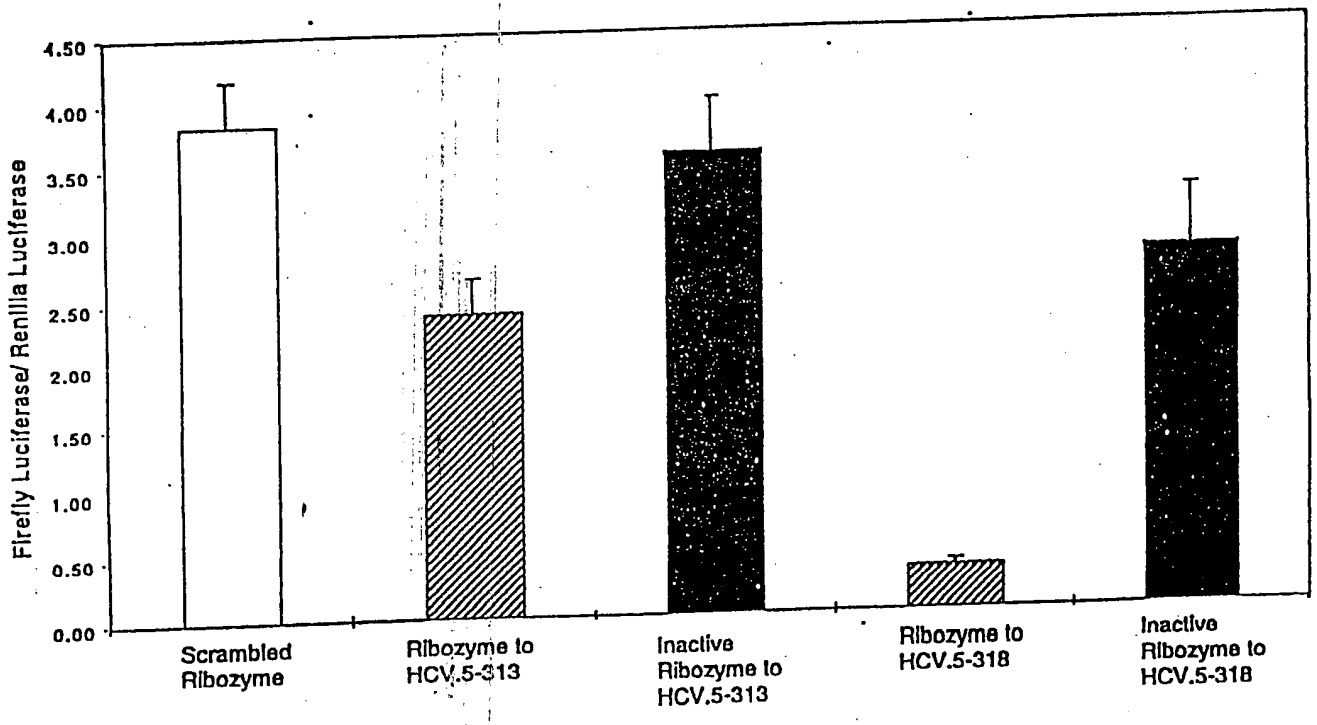
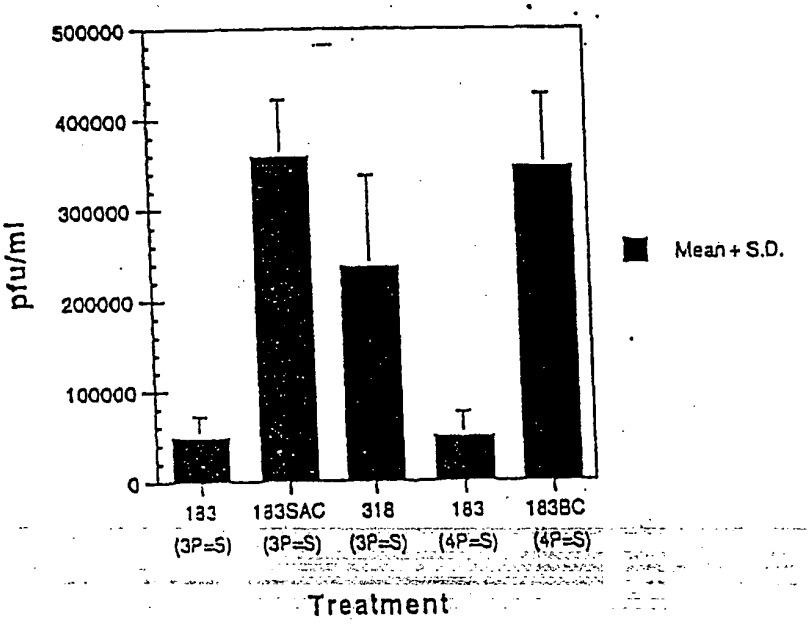
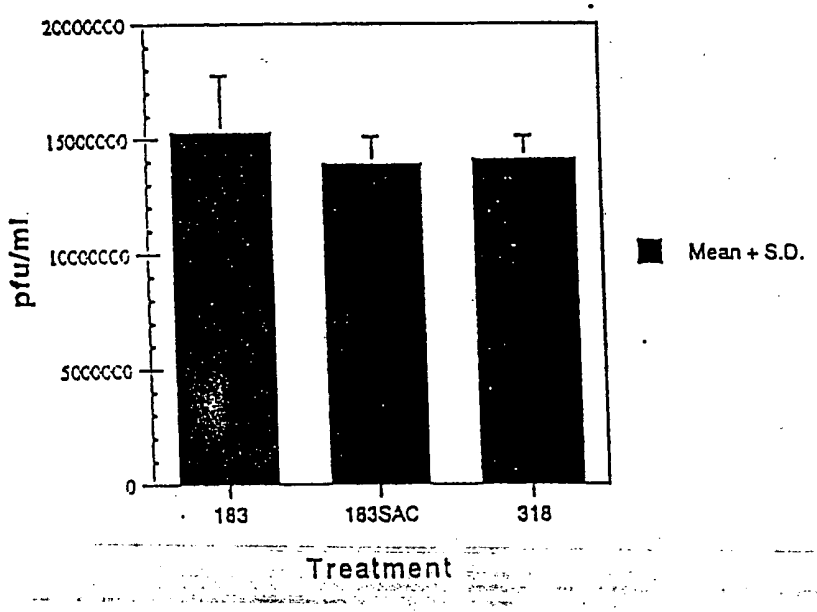


Figure 6A. Inhibition of HCV-Polio Virus Using Ribozymes



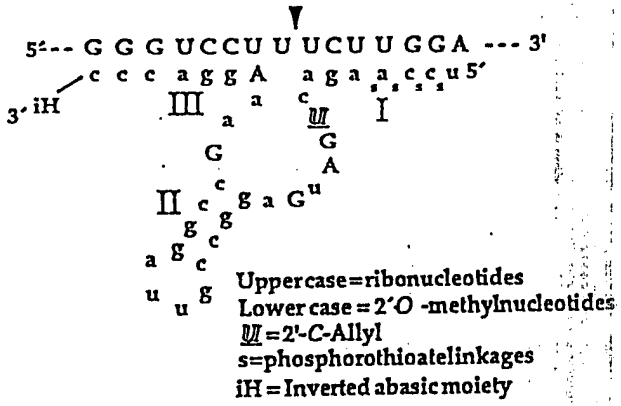
P=S-Phosphorothioate Internucleotide linkages at the 5' end
SAC-Scrambled Control
BC-Ribozyme with inactivated core

Figure 6B. No inhibition of Wild Type Polio Virus

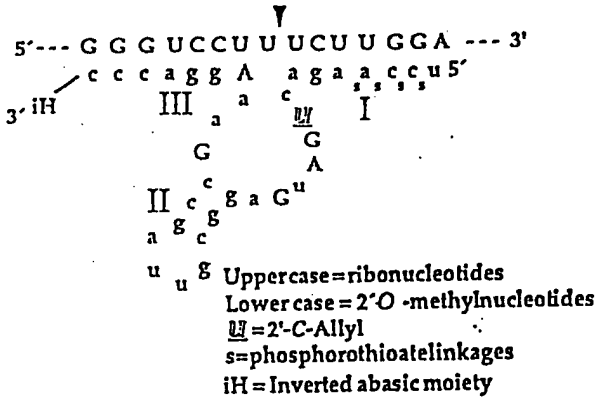


SAC-Scrambled Control

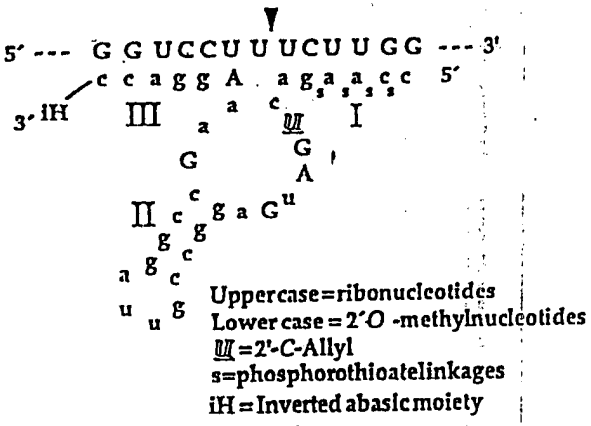
Figure 7 : Hammerhead Ribozymes Targeted Against HCV RNA



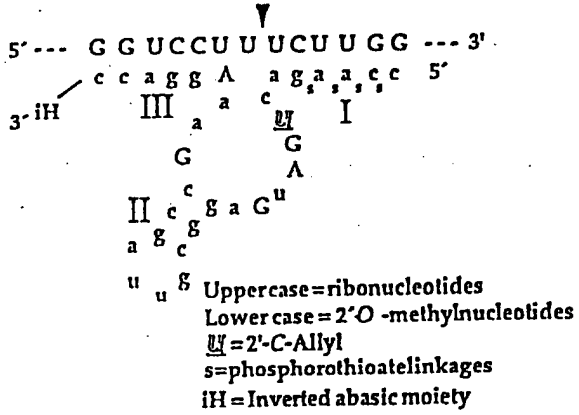
183 HH (7/4) Ribozyme



183 HH (7/3) Ribozyme

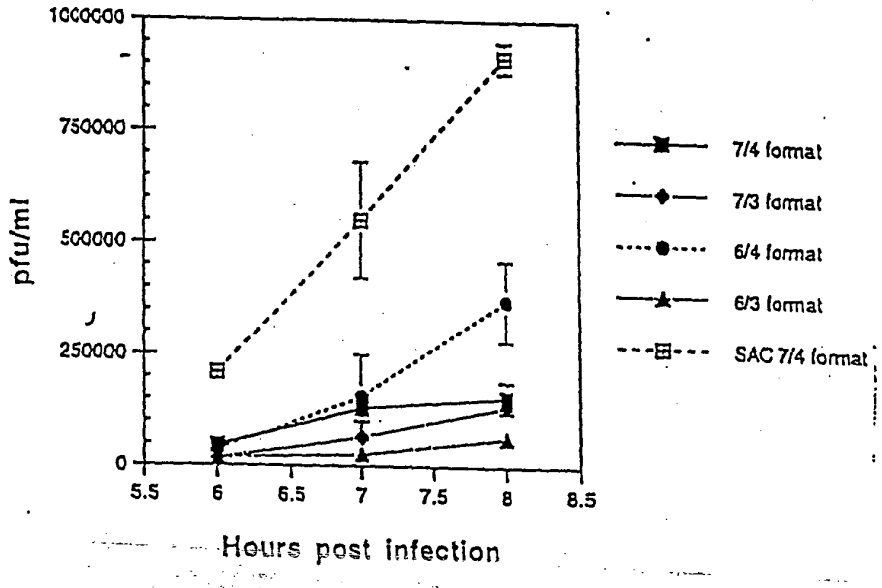


183 HH (6/4) Ribozyme



183 HH (6/3) Ribozyme

Figure 8. Inhibition of HCV-Polio Virus Using Ribozymes of varying lengths



SAC-Scrambled Control

Figure 9. Schematic Diagram for Ribozyme Motifs

241/078

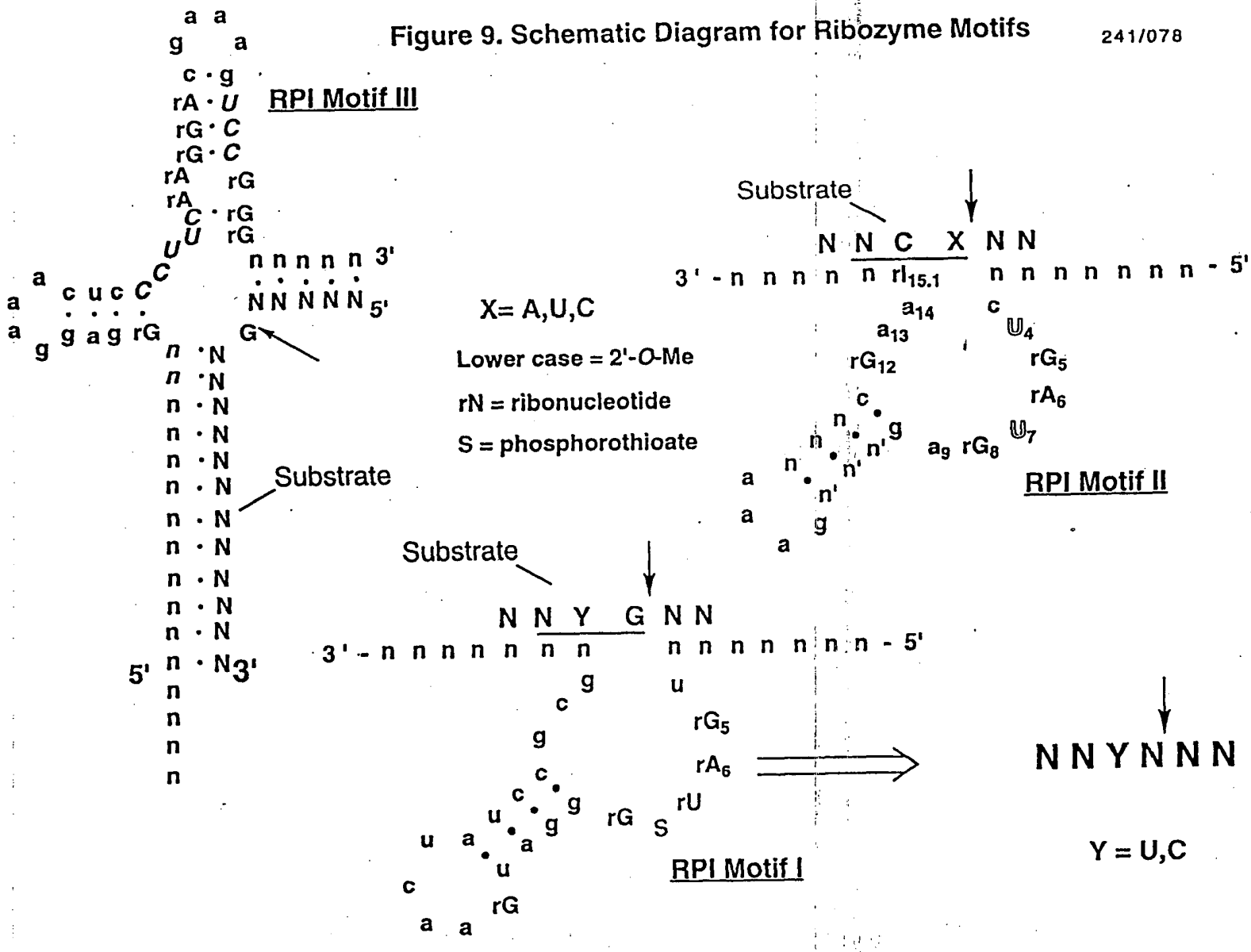


Figure 10: Synergistic effect of HCV Ribozyme and Interferon

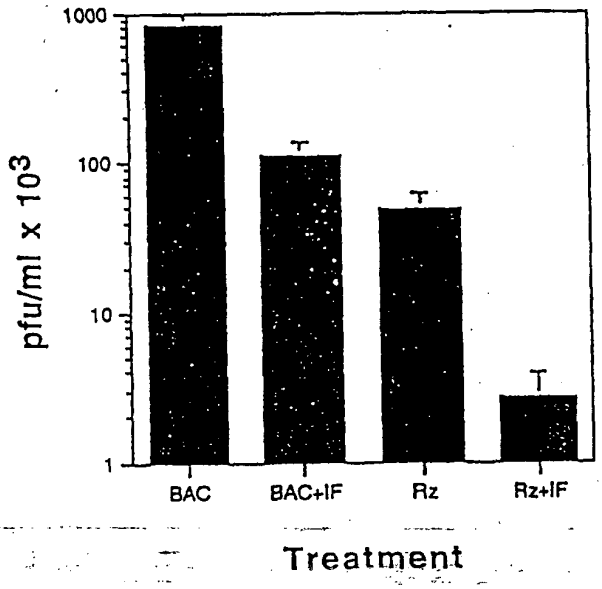


Figure 11: Screen of Anti-HCV Ribozymes Directed Against the Minus Strand

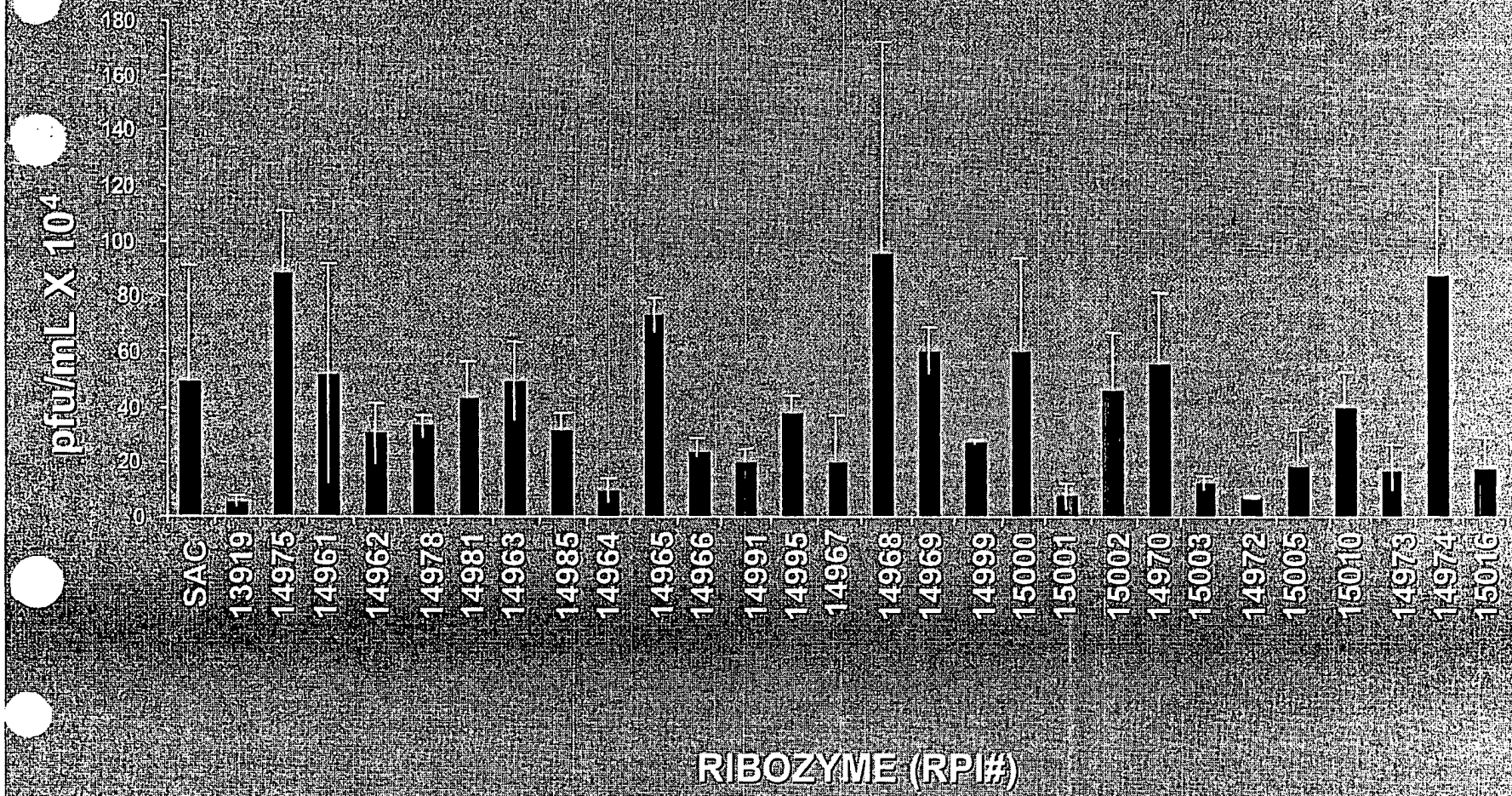


Figure 12: Additional Screen of Ribozymes Targeting the HCV Minus Strand

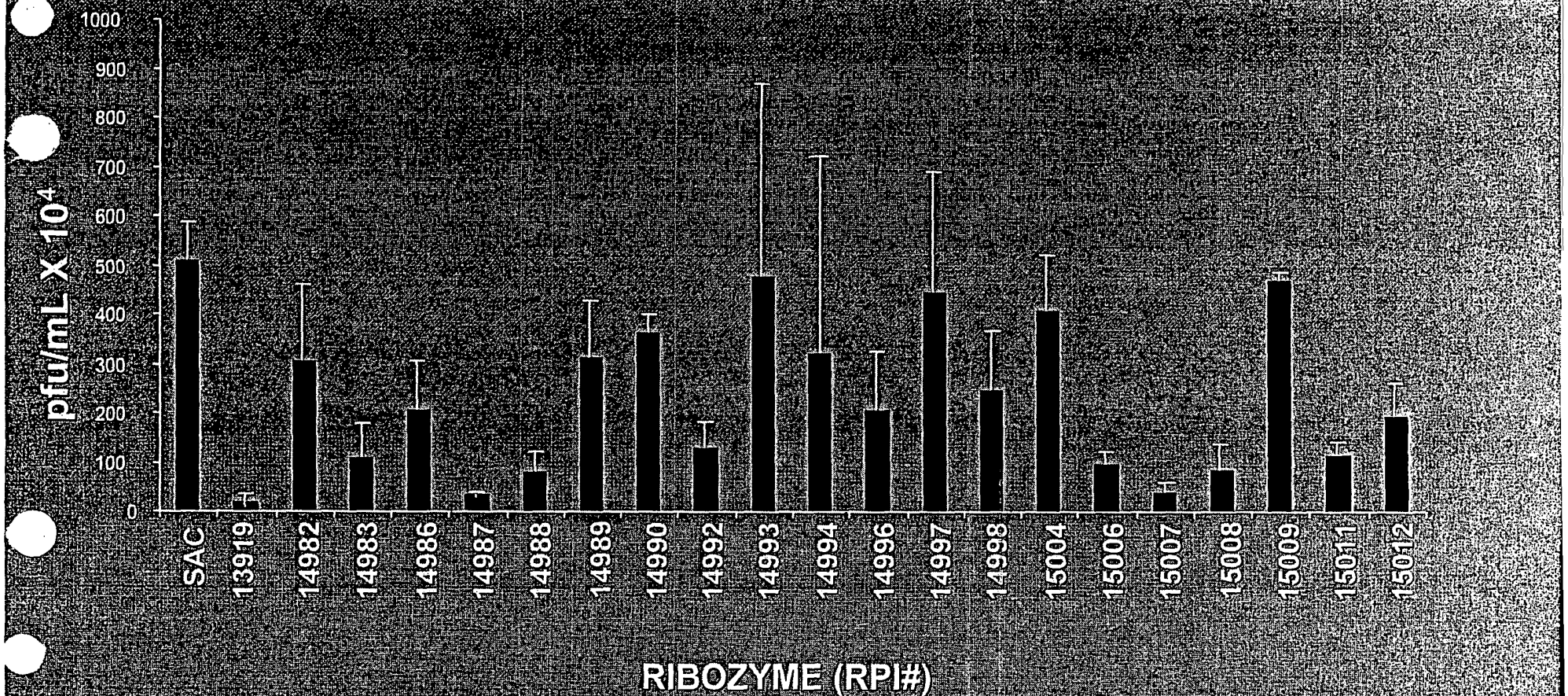


Figure 13: Dose Response for the RPI.15006 Anti-HCV Ribozyme

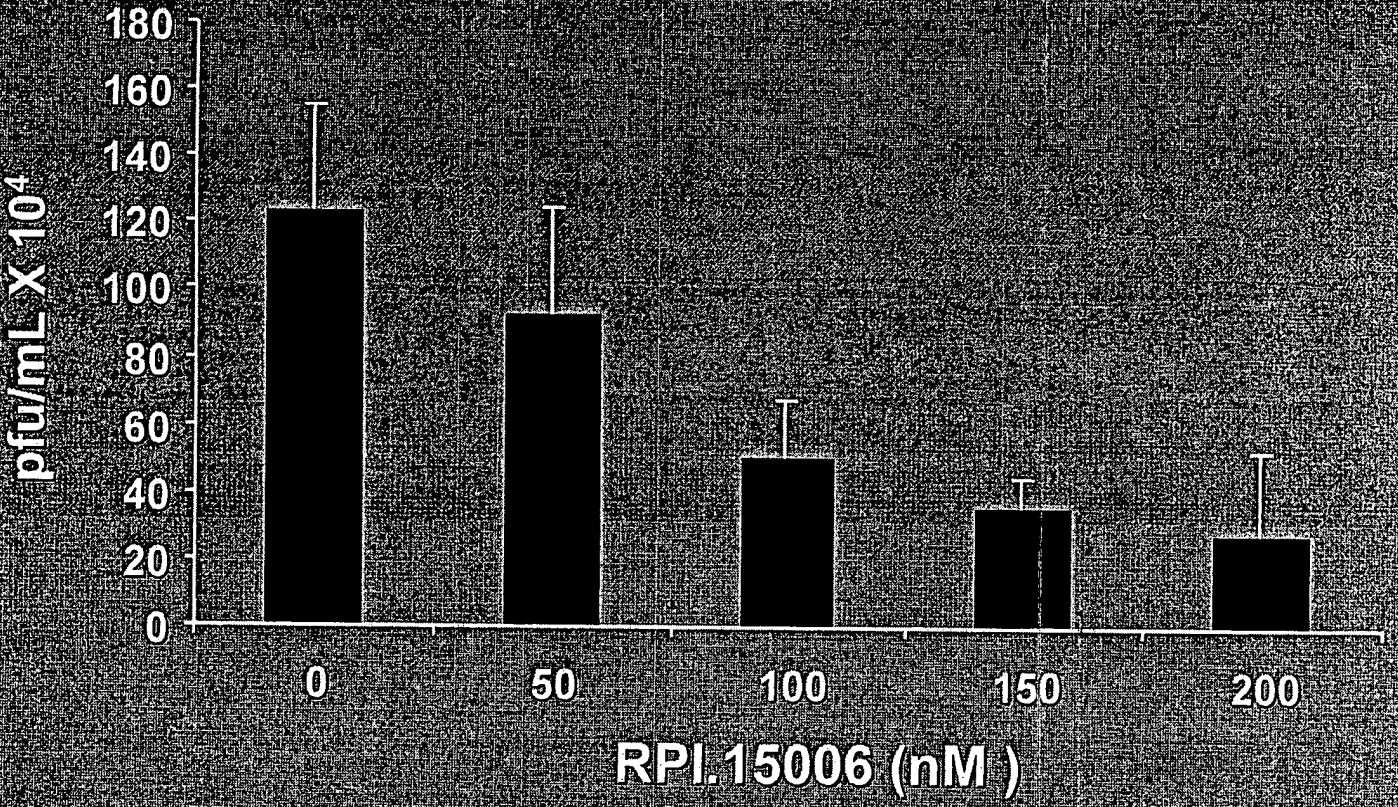


Figure 14: Dose response of Mixing Minus Strand Ribozymes and RPI.13919 Anti-HCV Ribozyme